OLUME 92

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NUMBER 3

BULLETIN of the JOHNS HOPKINS HOSPITAL

THE PUBLICATION OF THE MEDICAL SCHOOL AND HOSPITAL
SUPPORTED BY THE DE LAMAR FUND OF THE JOHNS HOPKINS UNIVERSITY

March, 1953

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PUBLISHED MONTHLY

THE JOHNS HOPKINS PRESS, Baltimore-18, Maryland

Made in United States of America

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Books for review should be sent to The Editor at the above address.

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Subscription price. In the United States and its possessions $6.00 per year. In Canada $6.25 per year; in foreign countries $6.50 per year. Prices for back volumes and back single numbers on application. Single numbers of the current volume, when available, $.75.

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THE JOHNS HOPKINS PRESS

BALTIMORE-18, MARYLAND, U. S. A.

Entered as second class matter at the Baltimore, Md., Post Office. Acceptance for mailing at special rate of postage provided for in Section 1103, act of October 3, 1917. Authorized on July 3, 1918.

RESPIRATORY EXERCISES FOR CHRONIC PULMONARY EMPHYSEMA

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Received for publication September 2, 1952

It is the purpose of this report to present a new technique in respiratory exercise which has recently been developed by the staff of the Physical Therapy Department of The Johns Hopkins Hospital for use in the treatment of patients with chronic emphysema.

The problems of faulty respiratory mechanics and inadequate ventilation with prolonged incomplete expiration have been recognized for many years. Treatment has usually been with abdominal belts, positive pressure, pneumoperitoneum, bronchodilators and similar agents. Alteration of the mechanics of breathing has been dismissed as unnatural and therapeutically impractical (1). To my knowledge the first suggestion that re-education of the respiratory cycle was a plausible approach to the problem was that of Thomas who advised "... training emphysema patients to start the respiratory cycle with forced expiration and allow inspiration to take care of itself" (2). Apparently there was no follow-up on his suggestion and it was not until 1949 in this clinic that specific efforts along these lines were made to devise an effective treatment for chronic emphysema. Weiser (3), however, reports only temporary improvement following his attempts to mobilize the diaphragm.

Prior to 1949 the exercises used with patients referred for respiratory exercises were in strict accordance with the procedure outlined by The Asthma Research Council (4) as a part of the Committee's report first published in 1935. Although their recommendations were directed toward the treatment of asthma it became the practice at this hospital to apply the regimen to patients with varying degrees of emphysema as well. During the last nine months of 1948 it was noted by the author that widely divergent results were obtained with patients referred for asthma exercises and these differences could not be explained satisfactorily on the basis of age, severity or etiology of the disease, past medical history or employment. A preliminary analysis of the patients treated during this period suggested that, with few exceptions, the benefits of treatment varied inversely with the degree of emphysema. Accordingly, a survey was made of all patients treated from May 1948 to July 1949. Groups were determined by diagnoses supplied by physicians and the results of functional studies.

Improvement is defined as increased ability to clear the tracheobronchial

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tree, decreased exertional dyspnea and increased exercise tolerance in routine activities. It is interesting to note that the decreasing percentage of good results parallels the increasing severity of emphysema. Approximately 80 per cent of patients with asthma only were improved as compared with 18 per cent of those who had only emphysema. The presence of even mild emphysema seems to have a marked effect on the value of the asthma exercises as applied to these patients.

The aims of the exercises recommended by The Asthma Research Council are two-fold: to clear the chest in the morning and night, and to abort or relieve attacks of asthma. These aims acknowledge the transitory nature of paroxysmal episodes of dyspnea due to uncontrolled asthma. On the basis of the contrasting characteristics of asthma and emphysema the problem seemed to be the formulation of a regimen of exercises specifically for patients with emphysema based on the peculiarities of that disease, particularly the constant nature of the disability and outflow obstruction, its permanence and progression. Since it is not possible to restore normal physiological properties to emphysematous lung tissue an alternative was considered—that of re-educating the breathing pattern in an attempt to improve

TABLE I
Clinical Results of Asthma Exercises
May 1948 to July 1949

GROUPS	TOTAL TREATED	IMPROVED		UNIMPROVED	
		Number	Per cent	Number	Per cent
A. Asthma only	10	8	80	2	20
B. Asthma, mild emphysema	6	2	33	4	67
C. Asthma, severe emphysema	17	4	24	13	76
D. Emphysema only	11	2	18	9	82
Total	44	16		28	

ventilatory mechanics. Outward clinical manifestations of emphysema are found in the habitual breathing pattern adopted by the patient in his natural attempt to overcome dyspnea. This effort is apparent in rapid shallow breathing, gulping inspiration and a chest maintained in inspiratory position. The new exercises seek to attack a permanent condition with constant therapeutic activity, emphasizing expiration as the active phase of respiration produced by forceful contraction of abdominal and external intercostal muscles, and inspiration as the passive phase. This pattern can also become habitual through constant practice and may be beneficial in proportion to the automaticity developed and the increase in the effectiveness of the ventilatory effort.

The differences between the old exercises for asthma and the new exercises for emphysema are fundamental. The Asthma Research Council recommends a short inspiratory sniff to minimize the amount of air brought into the lungs. This is an active inspiration which we have found undesirable when the patient is developing a new automatic breathing pattern. It also encourages arrythmic breathing typical of the patient with emphysema. Elimination of active inspiration is essential to mastery of the altered pattern in which passive inspiration is initiated by rebound from forceful expiration. It is for this reason that abdominal binders which limit abdominal motion in inspiration cannot be used in conjunction with this type of breathing.

The Council suggests that expiration be through pursed lips making a whistling sound and that it be continued until there is no more air and a wheezing sound is heard. It is our feeling that with the primary problem being outflow obstruction it is defeating our purpose to further inhibit the outward flow of air by voluntarily restricting the exit through which it must pass. In the emphysema exercises, air is allowed to flow through a mouth which is relaxed to an open position such as one might assume to speak an "Ah" without sound. This allows the use of the largest available respiratory outlet without developing tension at the throat. This principle is also the foundation for our opposition to the use of blow bottles which, in effect, increase outflow obstruction by presenting resistance to expiration. With reference to prolonged expiration, the asthmatics are encouraged to develop a sustained expiratory whistle for as long as 40–60 seconds. As with the inspiratory sniff, this cannot be incorporated into an automatic breathing pattern. The point of diminishing returns operates when the exertion required is out of proportion to the increase in ventilation which is achieved. This seems to be of particular importance in the presence of cor pulmonale. For this reason the patient may be taught to stop forceful expiration at a point short of completion but sufficient to promote good rebound for passive inspiration.

The Council recommends the reclining position only when the patient is "tight in the chest." In this position the hips and knees are bent, the head elevated and the trunk in flexion. It is essential that full advantage be taken of tension and power in abdominal muscles which are often relaxed and weak, and this position places them at an added mechanical disadvantage. Therefore, basic exercises are taught in anatomic supine position (i.e. head level with shoulders, hips and knees) and every treatment and practice period begins that way unless elevation of the head is necessary to relieve orthopnea or tension on the throat, or unless elevation of the knees is advisable to relieve tension on the anterior abdominal wall in the presence of hernia.

It has been found that the exercises outlined for asthmatics, though started gently, are often too vigorous for emphysematous patients who have either marked dyspnea or severe cor pulmonale. In these cases the first consideration in teaching the new type of breathing is to encourage control and decrease the rate of respiration. It may not be advisable to attempt further correction for some time if at all.

OUTLINE OF EXERCISES FOR EMPHYSEMA*

Indications

Dr. Richard L. Riley of the Department of Medicine was kind enough to make the following statement concerning medical and physiological indications.

"The indications for 'emphysema breathing exercises' are usually two: dyspnea on exertion and ventilatory insufficiency due to chronic generalized airway obstruction. The patients almost invariably hold their chests in the inspiratory position and have an increased antero-posterior diameter. Airway obstruction is more severe in expiration than in inspiration, and, when measured, the residual volume and ratio of residual volume to total capacity are found to be elevated. The maximal breathing capacity is invariably reduced. Physiological studies suggest that some of the patients have emphysema

*This outline in brief has appeared previously in a preliminary report to The American Clinical and Climatological Association in November, 1951 (5) and in The American Journal of Medical Sciences (6).

in the pathological sense with destruction of alveolar walls while others have primarily intrinsic disease of the airways. This distinction is often difficult if not impossible to make on clinical grounds and has not featured in the selection of patients for breathing exercises."

Aims

The specific aims of treatment are several, (1) to correct the patient's natural attempt to overcome exertional dyspnea which results in a breathing pattern in which gasping mouth breathing and spasmodic chest elevation serve to hyperinflate the chest and reduce expiratory effort, (2) to improve efficiency of air exchange within the limits imposed by degenerative changes already present in lung tissue, (3) to retard the rate of progression of the disease by correction of controllable factors of respiration which may encourage degeneration of lung tissue through stretching, (4) to teach coordination of a new breathing pattern with daily activities which will prevent or reduce exertional dyspnea, (5) to clear the chest of an accumulation of secretions which increase dead space and precipitate coughing, (6) to correct typical posture faults which accompany chronic chest disease and respiratory inefficiency, (7) to relieve tension and apprehension which are serious problems in themselves, (8) to teach the patient the importance of mastering the technique of breathing exercises.

As Wright has stated, treatment and prophylaxis are empirical since the underlying causes and basic mechanism for pathological dysfunction in emphysema are not completely understood (8). There is no set of exercises which can be mimeographed and a copy handed to a patient with instructions that they are to be done at home. The importance of individual differences and day to day developments cannot be overemphasized nor can difficulties and modifications be anticipated. Each patient is a different problem and specific prescriptions are made from day to day according to progress made and the barriers which develop. Individual instruction by experienced persons is imperative.

PROCEDURE AND PROGRESSION OF THE EXERCISES

I. Position—patient supine.
   A. Elevation.
      1. One pillow to relieve tension on the throat or minimum that will relieve orthopnea.
      2. Small pillow under knees if hernia indicates.

         NOTE: This is the position of least demand on the patient. There is full body support, complete relaxation is possible, body mechanicis is at its best, there is no tension on the throat, the chest and abdomen are free for motion and observation.

B. Orientation.
   1. Explain simply and clearly to the patient his difficulty on a mechanical basis, the procedure and aims of treatment.
   2. Teach him awareness of his respiratory habits and why they are wrong.
   3. Question closely to provide a baseline for evaluation.

C. Instruction.
   1. Teach essentials of corrected easy breathing.
      a. Air out through mouth.
      b. Air in through nose.
      c. Chest relaxed at resting level.
      d. Throat relaxed, air moving freely.
      e. Abdominal muscles contract to remove air from lungs.
      f. Abdominal muscles relax to allow air to return to the lungs.

         NOTE: This constitutes a complete reversal of both normal and faulty breathing patterns by making expiration the predominate work phase and allowing inspiration to be the rest phase as it occurs automatically, initiated by rebound of abdominal muscles as they relax at the end of expiration.

      g. Rate: Normal time for inspiration. Expiration lengthened to two or three times the inspiratory phase.
      h. Depth: Inspiration guided by expiration in direct proportion if relaxation is maintained. Expiration always remains accented to overcome hyperinflation and outflow obstruction.
   2. Deepen both phases by focusing attention on increased expiration maintaining all essentials.
   3. When 1 and 2 are mastered, add to abdominal expiratory pressure.
      a. Contraction and decrease of chest in all diameters.
      b. Lower rib retraction.

         NOTE: During inspiration, the chest returns to normal resting level by relaxation alone. At no time is it actively expanded.
   4. Teach C-1, 2, 3 in side lying positions, right and left.

      NOTE: No advancement to any new exercise or new activity should be made until all previous exercises have been mastered. This means that breathing can be maintained without dyspnea, tension or fatigue through 12–15 consecutive breaths.

      Care must be taken to employ the lower abdominal muscles to the same degree that upper abdominals function in expiration, and to keep the transition from one phase to the other smooth and rhythmic. Jerking, locking the breath, undue prolongation of expiration and tension all serve to create premature fatigue, dyspnea, and coughing. No automatic respiration can be developed without control.

      The point of fatigue must be the main consideration in determining treatment and practice periods. Fatigue results in inaccuracies, strain and loss of continuity essential to mastery of a new physical skill and its effective use.

II. Position—sitting.
   A. Good body mechanics.
      1. Head erect, neck not strained.
      2. Upper back straight.
      3. Arms hanging from shoulders, hands resting on thighs or bed.

4. Lower back straight, not rigid.
5. Knees apart to give stability.

NOTE: This constitutes a progression in activity. Meeting and overcoming gravity and maintaining balance and good posture with less supportive base demands more effort than the resting level as in I-A. Hip flexion in sitting reduces the ease with which the abdominal muscles can be used in expiration due to the approximation of their origins and insertions. The loss in tension must be overcome by active contraction up to the point from which further work can increase intra-abdominal pressure sufficiently to move air. Control of lower abdominals is more difficult and they must be made to supply their full share of power in expiration, otherwise, there is less than full utilization of motive force.

B. Teach easy breathing as in I-C-1.
C. Add I-C-2 maintaining all correction of previous steps.
D. Add I-C-3 usually an easy progression.

NOTE: It may be necessary at first, with severely disabled, non-ambulatory patients, to support the back, but sitting alone should precede graduation to the next exercise.

III. Position—standing (may use wall for support if necessary at first).

NOTE: Again, this is a natural progression. Elimination of one disadvantage in flexed hips is outweighed by the increased exertion necessary to support all the weight in an upright position and in good alignment upon a markedly reduced base.

A. Correct posture without adding tension.
B. Teach I-C-1, 2 and 3 progressing only as previous skills are maintained.

IV. Teach walking coordinated with easy rate of respiration.
Maintain good posture.

NOTE: This rate will vary from patient to patient.

V. Teach overcoming added resistance with coordinated breathing.
A. Lifting object to various heights.
B. Replacing objects at various levels.
C. Carrying objects of various weights to increasing distances.

NOTE: Begin with book, pillow, small stool, light chair gradually increasing work and distance. Weight is lifted, work is done during expiration, walking is done as in IV, object is replaced and the body returns to erect position during expiration. Easy performance with this coordination can be accomplished only with practice.

VI. Eliminate mouth breathing in expiration except during heavy exercise.
VII. Teach stair climbing. Rising 3–4 steps during expiration, 1 step with inspiration, keeping same ratio descending.
VIII. Translate all usual light activities into coordinated breathing pattern.
IX. Encourage gradual increase of walking and stair climbing as criteria of improvement of respiratory efficiency.
X. Optional according to general condition—Teach mild postural exercises all timed with corrected respiratory pattern.

GENERAL REMARKS

By the time the patient has progressed to the standing position some degree of automatic carry-over will have taken place, at least at rest and asleep. The extent of the carry-over will increase as time goes on until the original breathing pattern will be difficult to reproduce. It is a physical skill learned

by constant practice and attention to detail and, once mastered, becomes the easier way to breathe.

The patient is encouraged to practice 5–15 minutes per hour depending on his general condition and the time at his disposal. However, he must be cautioned not to allow determination to carry him to the point of undue fatigue.

Throughout the exercise periods, coughing and expectoration must be encouraged. There may be a marked increase in frequency of cough and production of sputum for a few days, or it may begin to decline immediately. If there is an accumulation of foreign material in the lungs and the exercises are being done correctly, it usually follows that an increase in production of sputum will occur, and the patient must be told to expect this and not allow it to worry him. It is a basic value of deep respiration as these patients are taught to do it.

The length of treatment under daily supervison depends entirely upon the patient, governed largely by the severity of his disease, the original degree of disability, his cooperation in every detail, practice time and treatment time and other factors which might influence his progress. Deafness, language difficulties, reduced mental development and emotional instability may contribute to an unsatisfactory result. This is a form of treatment which cannot be applied with any success without the full cooperation of the patient. Until he understands what is to be done and what we hope to accomplish and unless he learns to trust the radical change in his manner of breathing, he will not be interested in working for the proficiency from which improvement can be expected.

The concurrent use of medications and other procedures has often obscured the degree of immediate improvement which might be attributed to respiratory exercises. The only index we have is the continued improvement with the use of the exercises after other measures have been discontinued. Although hospitalization would seem to be preferred, good results have been obtained with out-patients.

EVALUATION OF METHOD

Any attempt at evaluation of this method of treatment must be made with recognition that the information is largely subjective and does not lend itself to laboratory confirmation at this time. Also that the improvement noted often was due in no measurable degree to the relief of apprehension and the development of a sense of security from being able to combat attacks of dyspnea and control their severity. The psychotherapeutic value of this treatment must not be underrated since the attitude of the patient toward his disability plays such an important part in the effectiveness of his treatment. The latter is dependent to a large extent on his ability to relax and follow instructions. Furthermore, no rigid clinical criteria of improvement were established and no systematic survey of pulmonary function was possible.

However, a second survey completed 1 November 1952 provides some indication of the value of the new type of exercise which has been taught since 1 July 1949. Table II is presented as a guide to evaluation and reflects the fact that the large majority of patients who received adequate treatment and followed the suggested program were able to increase their activity with less respiratory discomfort.

A large number of factors contribute to the results shown in Table II. Among these may be mentioned the rather wide range of pathological conditions treated, the concomitant use of other therapeutic measures, the exclusion of many patients who were unable to complete the course of breathing exercises, and the variable degrees of awareness to respiratory disability. Pathological conditions in addition to emphysema included three cases of bronchiectasis, two of silicosis and one lung abscess. Ten of the 16 failures in Group D were extremely far advanced cases of emphysema. Other therapeutic measures often used in association with the breathing exercises included antibiotics, bronchodilators, ACTH or cortisone, etc. Patients who were excluded because of inadequate training in breathing exercises included those who were unable to understand due to deafness or language difficulty as well as those who were unwilling to cooperate. Finally, the subjective improvement on which Table II is based depended in part upon the patient's particular threshold of dyspnea and also upon the "awareness" factor mentioned by Wright (9). It is apparent that Table II represents an over-all subjective evaluation of a rather heterogeneous group who had in common the presence of ventilatory disability as a result of asthma and/or emphysema. Breathing exercises are believed to have been an important factor in the extraordinarily high rate of subjective improvement.

TABLE II*

Clinical Results of Emphysema Exercises

July 1949 to November 1952

(Group A was treated with asthma exercises)

GROUPS	TOTAL REFERRED	COMPLETED TREATMENT	IMPROVED		UNIMPROVED	
			Number	Per cent	Number	Per cent
A. Asthma only	61	39	37	94	2	6
B. Asthma, mild emphysema	21	12	11	92	1	8
C. Asthma, severe emphysema	101	72	67	93	5	7
D. Emphysema only	174	131	115	88	16	12
Total	357	244	224		20	

SUMMARY

Observations and a survey of clinical results following the use of asthma

* Material assembled subsequent to date paper was originally submitted.

exercises for patients with all types of chronic non-tuberculous chest disease with airway obstruction suggested that this method of treatment was less beneficial to patients with emphysema. Therefore, exercises were developed specifically for treatment of emphysema. Taking advantage of the relative absence of inspiratory obstruction, the breathing pattern is altered by developing strength and control of abdominal musculature and re-education in its use to produce an active forceful expiratory phase of the ventilatory cycle. The amount of inflowing air in the succeeding passive inspiratory cycle is determined by the elastic recoil of the entire chest and abdomen. Reversal of emphasis serves to interrupt hyperinflation of the lungs and allow more complete air exchange.

We have found that this altered respiratory pattern can become automatic and habitual through practice and can be employed to combat exertional dyspnea.

The entire program was developed with the peculiar characteristics of emphysema as a guide. Treatment is given with the recognition that we are dealing with a chronic disease in which there is progressive irreversible loss of elasticity of lung tissue and that some degree of disability may remain after the patient has achieved maximal benefit from the exercise.

This treatment of emphysema has the following advantages over the asthma exercises previously used: There is a potentially permanent value due to the automatic carry-over which can occur, mobility of the chest and diaphragm are restored to a variable degree through emphasis on expiration, it is initially less vigorous and falls more closely within a practical breathing pattern for constant use, it can be used voluntarily as an immediate therapeutic procedure when dyspnea occurs or undue exertion is necessary, it employs no external agents which may be expensive and inconvenient and it is particularly applicable to patients with severe cor pulmonale because it can be graded within their limitations and still be of value.

APPENDIX

For the purpose of showing more specifically what is meant by determining improvement by subjective measurement and observation, the following have been selected from the cases treated who have had symptomatic improvement. These have come from various services in the hospital and there are both in-patients and out-patients included.

J. R. (JHH #366956). 61 yr. W. M. Superintendent of carpenter crew. Out-patient. Severe pulmonary emphysema and chronic bronchitis for five years. Patient subject to paroxysmal episodes of productive coughing which often caused loss of consciousness for brief periods. Diaphragms low and immobile, marked change of contour of thorax with increased A-P diameter. Sleep often interrupted by coughing, appetite poor, chronic constipation, chronic fatigue. Speech jerking and hesitant with gasps for air between every two or three words. Severe exertional dyspnea after 10–15 steps at good speed. Stairs practically impossible to negotiate. Elevation of three pillows necessary for rest. Discharge: After treat-

ment every other day for two weeks and six weeks at home. Report from physician: "Walking up to 16 blocks daily. Back to work. Marked increase in diaphragmatic excursion to two to three inches." Can ascend three flights of steps at slow speed. Sleeping well, constipation relieved, clearing tracheobronchial tree easily.

C. T. (JHH #572647). 63 yr. W. M. Statistician. Admitted to hospital with diagnosis of pulmonary emphysema, severe, with asthmatic bronchitis, duration four years. Essentially non-ambulatory, any amount of walking resulting in marked dyspnea. Negotiated one flight of stairs with many stops and much discomfort. Episodes of asthma severe, marked weight loss and debility. Fatigue threshold very low. Discharge: After treatment daily over three month period (less about three weeks altogether due to upper respiratory infections and other complications) marked improvement. Walking about at will, asthmatic attacks practically disappeared, appetite good, gaining weight. Nine month recheck: Working five or six hours each day, eating normally, regained all of weight, can walk eight blocks without dyspnea, and can climb three flights of stairs slowly without trouble.

W. H. (JHH #600286). 62 yr. W. M. Newspaper executive. Admitted to hospital with diagnosis of pulmonary emphysema, severe, duration five years. Marked exertional dyspnea and episodes of shortness of breath caused by as little exertion as taking off a coat. Confined to chair. Cough nearly absent, never productive, diaphragms low, immobile, and irregular in contour. Discharge: After daily treatment for two weeks, marked improvement in comfort and control of dyspnea. However, there was only moderate increase in guarded activity. Patient felt that mobility depended on regaining of weight and would not attempt to increase activity very much. Three month recheck: Considerable improvement, gaining weight, breathing well, up and about cautiously with little discomfort.

F. H. (JHH #599038). 65 yr. W. M. Railroad executive. Admitted to hospital with diagnosis of severe bronchial asthma with severe secondary emphysema for five years. In chronic mild respiratory distress and practically non-ambulatory. Severe cough frequently productive. Breathing and speech labored with all accessory muscles in full play. Diaphragms low and immobile. Discharge: After daily treatment for three weeks, marked improvement in respiratory capacity. Walking as desired without difficulty.

V. F. (JHH #547152). 75 yr. W. M. Active in many capacities for many years. Out-patient. Pulmonary emphysema, moderately severe, duration two years. Dyspneic after one block at moderate pace or one flight of stairs slowly. Sleeping poorly, depressed and apprehensive. Accepting retirement though capable of doing many things. Rapid gasping breath, extreme fatigue, slow, uncertain gait. Discharge: After ten days, four daily treatments and three on alternate days—very little discomfort, able to climb three flights of stairs slowly and walk slowly indefinitely. Sleeping better and in far better frame of mind. One month recheck: Has job as telephone operator, walking slowly two miles each day, climbing stairs and moving about at will.

B. P. (JHH #423507). 64 yr. C. M. Painter, on Medical Care program. Out-patient. Pulmonary emphysema, moderately severe with early cor pulmonale, duration two years. Marked dyspnea after walking one block or after one flight of stairs, stopping two or three times. Sleeping poorly, moderate orthopnea, only moderate increase in A-P diameter of chest but respiration very rapid. Discharge: Walking consecutively two flights of stairs, four and one-half blocks and two flights of stairs without discomfort except fatigue in legs. Sleeping well and gaining strength. Plans to go back to work immediately. (Doing physical labor three months after treatment.)

G. G. (JHH #605666). 43 yr. W. M. Engineer. Admitted to hospital with moderately severe pulmonary emphysema with symptoms for three years. Asymptomatic at rest, however exertional dyspnea follows rapidly upon activity. Can tolerate only one flight of stairs.

Diaphragms low, slight increase A-P chest diameter. Discharge: After three daily treatments there was marked symptomatic improvement. One month checkup: Has climbed 12 flights of stairs without difficulty, and has little limitation of normal activity.

D. Y. (JHH #555863). 40 yr. W. F. Hotel manager. Admitted to hospital with emphysema secondary to severe bronchiectasis and tracheobronchial diverticuli, symptoms for 15 years. Severe chronic productive cough, extreme orthopnea, 70 degree elevation necessary for rest or sleep. Activity produces immediately coughing and dyspnea. Sleeping poorly, greatly fatigued. Practically non-ambulatory. Speech hoarse. Discharge: One week daily treatment: Within three days elevation on one pillow only necessary. Cough had decreased to two or three episodes per day, easily productive. Patient walking to clinic without fatigue. Sleeping well with one pillow. Speech normal, continuous and clear. Re-admitted one year later—same difficulty as before. Stated she had felt so well she had neglected exercise. After three day refresher course, again much better.

E. H. (JHH #577895). 40 yr. W. M. Businessman. Out-patient. Three year history of emphysema, mild, with chronic bronchitis. Experienced moderate dyspnea after walking two blocks or one flight of stairs. To have surgery necessitating general anesthetic, and treatment was to prepare for this procedure, as well as for usual activities. Moderate dyspnea after walking two blocks and after climbing one flight of stairs. Easy fatigue necessitated numerous rest periods during a normal day's work. Discharge: After five daily treatments. Noticed less fatigue with increased activity. Returned two months later for surgery and had continued to improve until there was little limitation to normal activity.

ACKNOWLEDGMENTS

The author wishes to thank Dr. Donald F. Proctor for his guidance during the early days of the project, the staff of The Respiratory Laboratory for their advice and patient teaching and particularly Dr. E. M. Hanrahan without whose encouragement and very real help this paper would not have been completed. A group of the patients in this paper has been described by Dr. Warde B. Allen, with favorable comment as to the improvement they derived from the respiratory exercises. His help in continuing to refer patients for treatment is gratefully acknowledged.

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ALLERGIC REACTIONS TO THERAPEUTIC AGENTS: TREATMENT WITH ADRENOCORTICOTROPIC HORMONE (ACTH) OR CORTISONE

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Received for publication September 6, 1952

Since the previous communication describing the effects of adrenocorticotropic hormone (ACTH) and cortisone on drug hypersensitivity reactions of the serum-sickness type (1), a more extensive experience has been accumulated during which there has been an opportunity to compare the relative therapeutic merits of cortisone, hydrocortisone and ACTH given by various routes of administration. The response to treatment in the majority of the cases was prompt and complete, and the relief afforded in this type of abnormality represents one of the most practical clinical usages of these hormones. The allergen can usually be identified and withdrawn. The relief is prompt and the short course of treatment necessary is safe and relatively inexpensive.

In the present report the results of treatment of 24 patients with hypersensitivity reactions will be described—13 of the reactions were due to penicillin, eight to horse serum and one each to sulfonamides, para-aminosalicylic acid and gold. The most satisfactory results have been obtained in the eight cases treated with cortisone by mouth which produces a rapid response and is easy to administer. Five cases of sensitivity to ACTH also will be described briefly.

REACTIONS TO PENICILLIN

In the previous communication (1) the results during the treatment of six patients with hypersensitivity reactions to penicillin were described. Five patients received ACTH and one cortisone by intramuscular injection. Since that time other reports have appeared. Friedländer and Friedländer (2) described a dramatic response to cortisone by oral administration in two patients with severe reactions to penicillin manifested by urticaria and angioneurotic edema. Cases have also been reported by Feinberg, Dannenberg and Malkiel (3) and by Schwartz (4) in which an excellent response to hormone administration was obtained.

All 13 of the additional patients with sensitivity to penicillin to be described in the present paper had a severe reaction which was increasing in intensity at the time treatment was begun. The patients ranged in age from six to 55 years. Two had previously had a reaction to penicillin; two were sensitive to horse

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serum; three had a history of hay fever, one of asthma and one of urticaria of undetermined etiology.

In nine of the cases there was a definite history of the previous administration of penicillin; in the conjunctival sac, intranasally, cutaneously, and orally in one case each, and intramuscularly in the others.

The average time from the start of penicillin administration to the onset of the reaction was nine days with a range of one to 14 days. The average duration of the sensitivity reaction before treatment with ACTH or cortisone was 5.5 days. Most of the patients had other forms of therapy first. Of those who received large doses of pyribenzamine two noted a very slight beneficial effect; eight, none. Six were given epinephrine with little or no improvement. In one, treatment with procaine given intravenously had no benefit, and in another, benadryl failed to improve the patient's condition.

In the following table are listed the clinical manifestations which were observed in this group of patients:

Fever		13
Pruritus, generalized		13
Cutaneous lesions	urticaria	12
	vesicles	2
	exfoliation	2
	hemorrhagic	2
Angioneurotic edema		8
Conjunctivitis & palpebral edema		7
Generalized lymph node enlargement		5
Arthritis		4
Nausea and vomiting		4
Dyspnea and wheezing		2
Circulatory collapse		1

In several cases there was hoarseness and difficulty in swallowing. The usual picture was that of fever, extensive giant urticaria with erythema and angioneurotic edema.

In only six was the white cell count above 10,000 per cu.mm. In most instances the number of eosinophils per cu.mm. of blood was within the normal range; the highest count was 352.

The average total dose of cortisone administered orally was 760 mg. with a minimum of 350 and a maximum of 1050. In each case the initial dose was 100 mg. The average duration of treatment was 2.7 days with a range of one to five days. The dosage schedule varied. In general, after an initial dose of 100 mg., 50 mg. was administered every four hours for six doses and then 25 to 50 mg. every six hours for an additional day. In one severe case, after the first dose of 100 mg., 50 mg. was given every two hours for 12 doses, then 50 mg. every four hours for six doses. During the first day of treatment there was relief, usually within 30 to 60 minutes after a dose, with diminished pruritus and

fading of erythema and urticaria. Two hours after a dose the manifestations increased again in severity necessitating the short interval between doses. In other patients given the hormone at four to six hour intervals the improvement which took place uniformly after the initial dose was maintained. In one patient with early pneumococcal meningitis it was felt desirable to continue penicillin. All manifestations of the hypersensitivity reaction were controlled by 100 mg. of cortisone by mouth followed by 50 mg. every four hours for 48 hours during which time the patient was continued on 16 million units of penicillin daily. Cortisone was stopped after 48 hours and 24 hours later the fever, urticaria and angioneurotic edema reappeared. Penicillin was then stopped and aureomycin begun. The manifestations of the allergic reaction gradually subsided.

After the start of therapy with cortisone orally pruritus and nausea were improved or had completely abated within two to four hours in most cases. Urticaria and angioneurotic edema showed objective improvement within the same time period and were uniformly entirely absent in from 12 to 36 hours. In general, the patients were entirely comfortable after 12 hours of treatment and the allergic manifestations had subsided in 24 to 36 hours.

The intravenous infusions of ACTH were administered in 750 to 1000 cc. of 5% glucose over periods of eight to 12 hours. In one patient a single infusion containing 40 mg. of ACTH was given; in a second, 20 mg. daily for two days and 30 mg. on the third day; in the third case 20 mg. daily for four days. Improvement occurred 10 to 12 hours after the first infusion. A mild recurrence was then noted in two of the cases six to 12 hours later which was more promptly relieved after the start of the second infusion.

In the patient treated with ACTH intramuscularly there was an incomplete response during four days of treatment with 25 mg. every four hours. The dose was raised to 35 mg. every four hours for one day which produced a satisfactory eosinophil response and complete relief. The total daily dose was then lowered by 20 mg. every other day, stopping the hormone when a daily dose of 40 mg. was reached.

The patient treated with repository ACTH (in Armour's Adactar Vehicle-32, containing polyvinyl-pyrrolidone) was given 100 mg. every 12 hours for four doses and then 50 mg. twice daily for two days for a total dose of 600 mg. Nausea and pruritus were improved within eight hours after the first dose and essentially complete relief was noted in 24 hours.

In one case 50 mg. of Compound F-acetate was given intramuscularly every 12 hours for three days and a single dose of 100 mg. on the fourth day. The eosinophil count, which was 352 per cu.mm. initially, fell to 55 on the third day of treatment and rose to 187 on the day after the last dose of hormone. Improvement was slow during the first two days but progressed rapidly when the eosinophil count reached a low level.

Recurrence after stopping treatment occurred in two patients given ACTH

intravenously and in two other patients given cortisone orally. In two of these patients, one given ACTH over seven days and the other cortisone over two days, the manifestations of the sensitivity reaction recurred promptly, nine and 28 hours, respectively, after the hormone was stopped. In both patients the administration of penicillin had been continued both during and after hormone treatment. When the manifestations of the allergic reaction reappeared the penicillin was discontinued immediately. In the patient given ACTH the urticaria continued unchanged during the next 48 hours in spite of repeated doses of pyribenzamine. ACTH was then readministered and the urticaria subsided within 24 hours. After two days the ACTH was again discontinued and there was no recurrence. In the patient given cortisone the reaction gradually cleared over a period of four days without further treatment.

In the other two patients the recurrences were more delayed, both occurring five days after stopping hormone administration. In these patients treatment with penicillin had been discontinued prior to hormone administration. In the patient given ACTH intravenously over a period of five days, mild arthralgia lasting only one day was the sole manifestation of the recurrence. In the other patient who received cortisone orally for three days the recurrence was sufficiently severe to warrant retreatment. Cortisone was given again orally for two days. There was prompt clearing of the urticaria within 24 hours and the patient remained asymptomatic thereafter.

The following case report will serve to illustrate the effect of the administration of cortisone in a severe sensitivity reaction to penicillin:

J. H. (JHH No. 560,757) This 34-year old white public relations officer entered the hospital on January 10, 1951, because of severe generalized urticaria.

Two and one-half years previously the patient was given penicillin aerosol for an upper respiratory infection. Three days later he developed inflammation and swelling of the left eye and for this penicillin ointment was prescribed. The ocular inflammation became more severe and the entire left side of the face became swollen. After discontinuing the penicillin ointment, the reaction slowly subsided over a period of a few months. The patient does not recall having received penicillin at any other time.

Twenty-four days prior to admission he developed a "virus infection" during which he received three daily injections of penicillin-in-oil intramuscularly. Ten days prior to admission he noted a swollen, erythematous, pruritic area over the left ankle with the rapid appearance of similar lesions over the rest of the body. Pyribenzamine, 200 mg. daily, afforded slight relief from the itching, but no objective improvement. Procaine given intravenously, five days later, was similarly ineffective. The edema increased and he noted shortness of breath. The following day, four days prior to admission, aqueous epinephrine subcutaneously and epinephrine-in-oil intramuscularly effected reduction in the urticaria. However, the generalized eruption reappeared on the day before admission and was unresponsive to pyribenzamine and both preparations of epinephrine. The morning of admission, the "welts" and itching became severe, he felt feverish, and noted arthralgia in the left knee. The tongue became swollen to the extent that it interfered with precise articulation. He developed a sore

throat with dysphagia and hoarseness. The shortness of breath recurred and nausea vomiting, diarrhea and mild abdominal cramping pain were noted.

On admission the blood pressure was 100/70 mm.Hg. He exhibited fever (102.0°F. rectally), tachycardia (132/min.) and tachypnea (28/min.) There was severe, generalized, coalescing urticaria. The nail beds were slightly cyanotic. The sacrum and scrotum were edematous. There was injection of the conjunctival vessels. The tongue was swollen.

Aqueous epinephrine (1:1000), 0.6 ml., given shortly after admission, afforded no relief and he was started on 100 mg. of cortisone (Merck) orally at six hour intervals. This dosage schedule was maintained for 36 hours, then reduced over a 24-hour period to 25 mg. every six hours, at which level it was maintained during the next two days, a total dose of 1050 mg. being given over a five day period.

Within two hours after the first dose the patient noted improvement in that he felt stronger, the tongue became less swollen and the nausea and abdominal pain disappeared. Fourteen hours after the beginning of therapy, the patient having received 300 mg. of cortisone, the temperature was 98.6°F., the pulse rate 84/min., the respiratory rate 18/min., and the blood pressure 135/80 mm.Hg. The skin lesions had cleared dramatically, only blotchy slightly erythematous patches remaining over the anterior chest, forehead and hands. Sacral and scrotal edema, as well as arthralgia, were no longer present.

The remainder of the patient's course was uneventful except for occasional episodes of mild, transient urticaria appearing four to five hours after a dose of cortisone.

SERUM SICKNESS

Of the eight patients with serum sickness, seven followed the administration of tetanus antitoxin and one diphtheria antitoxin. Their ages ranged from 19 to 40 years. Six had previously received an injection of horse serum while in the other two there was no history of a sensitizing dose. The average time from the administration of the serum to the onset of the sensitivity reaction was four days, ranging from three to nine days. The duration of the reaction before hormone treatment was instituted was on the average two days with a range of two hours to five days. The manifestations of serum sickness were severe in all cases. The frequency with which the various subjective and objective findings were noted is given in the following table:

Fever	3
Pruritus	8
Urticaria	8
Local edema and urticaria at injection site	3
Angioneurotic edema	7
Generalized lymph node enlargement	6
Arthritis	3
Nausea and vomiting	4
Dyspnea	3
Hoarseness and dysphagia	2
Circulatory collapse	1

Three patients first received treatment with pyribenzamine and three others with epinephrine but no satisfactory improvement resulted.

Four of the patients were treated with ACTH given intramuscularly. The

total dose varied from 162 to 650 mg., averaging 300 mg. The initial dose varied from 25 to 50 mg. The average duration of treatment was three days, the shortest being two and the longest six days. One-fourth of the total daily dose was given every six hours. Three patients were treated with ACTH by intravenous infusion. Two received 40 mg. over a 10 hour period and the other 50 and 25 mg. on successive days. In the one patient treated with cortisone orally the initial dose was 100 mg. followed four hours later by 100 mg., then 50 mg. every four hours for six doses and 25 mg. every four hours for three doses.

There was a prompt and satisfactory response to treatment in all of the cases. In those given ACTH intramuscularly there was improvement in pruritus and urticaria with lessening of angioneurotic edema within six to eight hours after the initial dose and no further lesions formed. All evidences of the sensitivity reaction disappeared within 24 to 48 hours. In the two receiving ACTH intravenously there was dramatic clearing of the lesions in the 10 to 12 hours after starting the infusion. In the one case treated with repository ACTH (in Armour's Adactar Vehicle-32) improvement occurred with essentially the same speed as with ACTH in saline. In the case treated with cortisone by mouth striking improvement was noted within one hour after the initial 100 mg. dose. Then, one hour later, the lesions increased in severity. One hour following the second 100 mg. the same dramatic improvement was noted. The following summary of this case will serve to illustrate the type of patient chosen for treatment and the pattern of response:

E. C. (JHH No. 359,997) This 34-year old white male painter appeared on Feb. 17, 1951, complaining of generalized hives, faintness, sore throat and nausea of approximately 12 hours duration. Seven days previously, he had received 1500 units of tetanus antitoxin following removal of a splinter from the right hand.

Six and four years previously the patient had been given tetanus antitoxin. Five days after the second administration he noted itching at the site of the antitoxin injection and on the following day developed generalized urticaria, vertigo, nausea and vomiting. He was treated with aqueous epinephrine and benadryl with initial improvement, but it required approximately one week for the reaction to subside completely.

Examination on the present admission revealed the patient to be in acute distress with pruritus, generalized urticaria, and patchy erythema with multiple excoriations. The lips and eyelids were very swollen and he was hoarse. The vital signs were all within normal limits.

At 1:00 p.m. the patient was given 100 mg. of cortisone by mouth. One hour later, many of the urticarial lesions and almost all of the areas of erythema had disappeared; the remaining urticarial lesions were smaller and less erythematous. There was no pruritus. The hoarseness abated to some extent and the lips and eyelids appeared less edematous. One hour later, however, his status reverted to that on admission, where it remained for the next two hours. Four hours after the first dose, he was again given 100 mg. of cortisone orally with the same sequence of events; namely, almost complete clearing at one hour followed by almost full exacerbation of the reaction at two hours.

At 9:00 p.m. he was started on 50 mg. of cortisone orally every four hours for six doses, then 25 mg. every four hours for three doses. Twelve hours after the first dose (1:00 a.m. on

Feb. 18, 1951), the skin was normal in appearance; the hoarseness had disappeared; and there remained only slight edema of the lips and eyelids. After 24 hours this last manifestation of his allergic reaction had disappeared. There was no recurrence when the administration of cortisone was stopped.

It is well known that involvement of the nervous system may occur during the course of serum sickness. This may develop within a few hours after the onset of serum sickness with neuritic pain which is severe in nature and may be followed by motor paralysis, atrophy, loss of sensation and reflex activity. The neurological signs, of course, depend on the segments which are involved; but a common site is the brachial plexus, particularly the fifth and sixth cervical roots (5). Although most of the patients recover, this may be very slow and a significant proportion of patients are left with residual weakness and atrophy. The use of cortisone or ACTH would seem particularly indicated in a patient with serum sickness who develops any evidence of nervous system involvement. For this reason it seems worth while to describe briefly two cases in the present series who exhibited neurological manifestations:

M. D. (JHH No. 570,855) was a 36-year old white male who had received an injection of tetanus antitoxin in childhood. Eight days before admission on May 8, 1951, he received a second injection following treatment for a minor laceration. Three days later he developed serum sickness. The manifestations increased in severity over the ensuing five days.

On admission the patient's temperature was 101.8°F., and the pulse rate, 100 per min. The conjunctivae were injected. There was a generalized erythematous eruption and giant urticarial lesions were scattered over the entire body. There was generalized enlargement of the superficial lymph nodes, some being two cm. in diameter. They were tender to palpation. The manifestation which caused the patient the greatest discomfort was severe burning pain along the distribution of the sicatic nerve on both sides. The reflexes were hyperactive.

The total white cell count was 13,300 per cu.mm. with 3% eosinophiles. The spinal fluid was under normal pressure. Examination of the fluid showed 5 mononuclear cells per cu. mm., 4+ Pandy and 100 mg. protein per 100 ml.

The diagnosis was acute sciatic neuritis associated with serum sickness. ACTH was given intravenously; 40 mg. in one liter of 5% glucose being infused over a period of 12 hours. Within three hours after the infusion was begun there was diminution in the pain and at six hours it had almost entirely disappeared. Within 12 hours the tenderness on palpation of the lymph nodes had disappeared, erythema was no longer apparent, and only the ghosts of the urticarial lesions could be seen. Cerebrospinal fluid examined the following day had a protein content of 50 mg. per 100 ml. There was no recurrence of pain following the single intravenous injection of ACTH.

The second patient was M. J. (JHH No. 586,785) a 26 year old labor foreman, admitted on Oct. 14, 1951, because of drowsiness, severe headache, cervical rigidity and urinary retention. Nine days previously he had received 1500 units of tetanus antitoxin because of a laceration of the right hand. He had received tetanus antitoxin following an injury several years previously.

Two days after the repair of the laceration he noted redness and swelling around the site of the antitoxin injection. A few hours later he developed general malaise, nausea, vomiting

and shaking chills followed by fever and a constant occipital headache. These symptoms continued in varying degree over the next three days. During this period he developed a generalized erythematous eruption and periorbital edema. On October 10 he was given aqueous epinephrine and pyribenzamine with transient clearing of the eruption. The following day he developed frontal headache, stiffness of the neck, transient episodes of diplopia and severe pain in the muscles and joints. These persisted and two days later he could not void in spite of a sensation of fullness in the bladder. On examination the bladder was palpated at the umbilicus and on catheterization 1050 cc. of urine was obtained. Twelve hours later he still had not been able to void and was admitted to the hospital.

On admission the patient's temperature was 101°F (rectal) and the pulse rate, 110 per minute. The patient appeared acutely ill, was drowsy and lethargic. He complained of severe pain in all of the muscles and joints. There was erythema around the site of the antitoxin injection and over the face and neck. The conjunctivae were injected. Funduscopic examination revealed blurring with elevation of the optic disc on the left and slight blurring of the disc margin on the right. There was rigidity of the neck. Muscle strength was generally reduced. The deep tendon reflexes were equal and active except for diminished biceps and triceps response on the left.

The white cell count was 14,900 per cu.mm. with 5% eosinophiles. Examination of the cerebrospinal fluid showed it to be under normal pressure with 50 mg. per 100 ml. protein and 18 cells per cu.mm., all mononuclear.

On the night of admission he was given 40 mg. of ACTH intravenously over a 10-hour period. This was repeated daily for the next four days and the dose was gradually reduced over the next three days. By the end of the first infusion the temperature and pulse rate had decreased to normal. The patient stated that he felt better generally and the pain in the muscles and joints had disappeared. There was less weakness of the muscles and less cervical rigidity. However, he remained somewhat lethargic. All of the tendon reflexes were equal and active. Improvement continued during the next two days and he was then walking about the ward without any symptoms or signs. Neurological examination was entirely normal including the appearance of the optic discs. On this day he was able to void spontaneously for the first time. After the second infusion of ACTH the lumbar puncture was repeated and the cerebrospinal fluid removed had a protein concentration of 25 mg. per 100 ml. and eight mononuclear cells per cu.mm.

It is impossible to predict what might have happened in either of these cases if treatment with ACTH had not been instituted. However, in view of the frequency with which persistent and disabling disturbances in function may appear following involvement of the nervous system in the course of serum sickness (5), the presence of signs or symptoms suggesting such involvement are indication for the administration of either ACTH or cortisone.

OTHER DRUG REACTIONS

A satisfactory response has been obtained in the treatment of a severe reaction to sulfonamides characterized by fever and arthritis, a febrile reaction to para-aminosalicylic acid (PASA), and a persistent exfoliative dermatitis due to gold which had been treated unsuccessfully with British antilewisite (BAL). These cases will be summarized briefly:

A 32-year old Negro housewife (JHH No. 511,749) was given triple-sulfonamide tablets over a period of ten days because of a severe respiratory tract infection. Five days after stopping the sulfonamides she developed chills, nausea, fever, and a severe polyarthritis. These manifestations progressed in severity despite large doses of salicylates. Four days later her temperature was 102.4°F (R). There was generalized erythema and a severe polyarthritis with slight generalized lymph node enlargement. The white cell count was 24,000 per cu.mm. with 660 eosinophils per cu.mm. She received an initial dose of 50 mg. of pork ACTH by intravenous infusion over a period of 10 hours. Five days of treatment were given, the dose being reduced by 10 mg. each day. She was afebrile after the first infusion and completely free of symptoms at the end of 48 hours. The eosinophil count fell to 55 per cu. mm. on the second day. There was no recurrence.

The next patient was a 17-year old colored boy (JHH No. 341,624) with disseminated tuberculosis. On the eighth day of treatment with streptomycin and PASA he developed a shaking chill with rise in temperature to 105°F. The PASA was omitted for one day and the temperature decreased to normal. When it was resumed the following day a febrile reaction developed. He was then given promizole for the next ten days at which time a similar febrile reaction appeared. The PASA treatment was then resumed and he was given a daily intravenous infusion of 30 mg. of ACTH for the next six days. No febrile reaction appeared. On the second day of ACTH the eosinophil count had dropped from 1397 to 11 per cu.mm. The skin reaction to old tuberculin, strongly positive prior to ACTH, was not elicited during ACTH. After six days the ACTH was stopped but PASA was continued and on the following day the high fever reappeared. No further ACTH administration was thought justifiable because of the possible deleterious effects on the course of his tuberculosis.

The third patient was a 63-year old white woman (JHH No. 558,114) who had rheumatoid arthritis and a dermatitis following gold therapy. After the administration of BAL the cutaneous lesions improved temporarily, but at the time of admission to the hospital there was generalized dryness and scaling of the skin with numerous weeping areas on the back, knees and breasts. She was given ACTH intramuscularly over a period of 19 days. After 10 days on 100 mg. the dose was gradually reduced, the total amount administered by the completion of therapy being 1.5 gms. Her skin showed improvement within 48 hours, and before termination of treatment healing had occurred leaving some residual pigmentation. After termination of treatment there was a return of the arthritic manifestations but no relapse of the dermatitis.

Steinberg and Roodenburg (6) have recently reported the use of ACTH in a case of dermatitis which developed during the use of myochrysine. Treatment with gold was discontinued. ACTH was given in moderate doses over a period of two weeks. The dermatitis cleared completely and there was no recurrence when administration of the hormone was discontinued.

SENSITIVITY TO ACTH

Shortly after the release of ACTH for clinical investigation in 1949, hypersensitivity reactions to it were reported. At the first ACTH conference in October of that year Trager (7) reported an allergic reaction following ACTH administration in four of 19 patients. At the second conference in December,

1950 Brown and Hollander (8) reported that a hypersensitivity reaction to pork ACTH developed in seven of 208 patients receiving the hormone. This was apparently a species sensitivity as several of the patients were subsequently given beef ACTH without reaction. In discussing this paper Stevenson reported the development of a sensitivity reaction in six of 113 patients treated with the hormone. In discussing the same paper Sauer described a patient with exfoliative dermatitis which seemed due to sensitivity to ACTH. The patient was placed on cortisone 100 mg. twice daily. It was the impression that during the six days of cortisone therapy the reaction to ACTH was suppressed and after discontinuing it relapse occurred with reappearance first of erythema and then exfoliation.

During the past three years over 600 patients have been treated with ACTH in The Johns Hopkins Hospital, many of them receiving multiple courses of the hormone. Fifty have received beef ACTH, seven sheep material, and the remainder porcine hormone. In only five cases has a definite hypersensitivity reaction been observed. In two cases the sensitivity was to pig material, in two to beef material, and in the other to hormone derived from both sources. A brief summary will be given of each of these cases:

The first patient (JHH No. 148,326) was a 43 year old white male who had had severe intractable headaches for nine years. In February, 1951 he received 140 mg. of ACTH (pork) daily by intravenous injection for five days and then at home 100 mg. of cortisone orally daily for one week. There was temporary subjective improvement. In June, 1951 he was started on daily intravenous infusions of ACTH (pork) and histamine. On the 13th day he received 20 mg. of ACTH and 1.0 mg. of histamine base over a period of eight hours. A few hours later he awoke with generalized urticaria and angioneurotic edema of the lips, periorbital tissues and genitalia. The following day he received ACTH alone which was followed by a similar reaction. The next day, six hours after beginning an infusion of ACTH (10 mg.) and histamine, he developed a severe reaction. It was thought that the reaction was due to the histamine so that the intravenous injection of ACTH was resumed. Two hours later the lesions were receding, but at three hours they increased rapidly. The infusion was stopped and the reaction became progressively more severe over the next 24 hours with dysphagia and hoarseness. Pyribenzamine afforded no relief. He was given an initial dose of 100 mg. of cortisone orally followed by 50 mg. every four hours for six doses, every six hours for eight doses and 25 mg. every six hours for four doses. There was a striking improvement within six hours after the first dose and complete healing at the end of 24 hours.

The second patient (JHH No. 558, 900) was a 52-year old white male who had chronic dermatomyositis. He received pork ACTH intramuscularly in doses varying from 100 to 140 mg. daily for ten days. He was then given beef ACTH. On the third day his temperature rose to 101°(R) and a maculo-papular eruption appeared which rapidly became confluent. Treatment with pork ACTH was resumed and the sensitivity manifestations rapidly disappeared. One week later, after ACTH had been discontinued, he had immediate and delayed reactions to both pork and beek ACTH in very dilute solution injected intradermally. Two days later after 10 mg. of beef ACTH injected intramuscularly he had a severe chill with a rise in temperature to 104.8° (R). The fever persisted for two days, but no eruption devel-

oped. Six days later he was given 10 mg. of pork ACTH intramuscularly and no reaction followed. He was then placed on cortisone by mouth, 50 mg. every four hours. Twenty-four hours later the injection of beef ACTH was repeated and no reaction followed. Twenty-eight hours after the last dose of cortisone the injection of beef ACTH was repeated and the temperature rose to 102°.

The third patient (JHH No. 105,508) was a 14-year old colored girl with sarcoidosis. In October, 1950 she was treated with 100 mg. of beef ACTH for 10 days and the dose was gradually tapered over the succeeding 10 days. In April, 1951 therapy was started with beef ACTH (30 mg.) given intravenously daily over a 10 to 12 hour period. Following the 14th infusion she developed headache and fever lasting for eight hours. The next day she was given 10 mg. of beef ACTH intramuscularly following which there was no reaction. Ten days later due to an exacerbation of her sarcoid lesions intravenous treatment with beef ACTH was resumed. Within 30 minutes generalized pruritus developed followed by angioneurotic edema and a maculo-papular eruption. The ACTH infusion was stopped. Within one hour the eruption was fading, the edema subsiding, and both were gone at the end of 12 hours.

The fourth patient (JHH No. 524,037) was a 35-year old colored male also with sarcoidosis who was admitted in March, 1952 for a third course of ACTH. In February, 1950 he had been given pork ACTH daily intramuscularly for seven days, and in May 1951 pork ACTH intramuscularly for seven days and then intravenously for 17 days. At 12:45 p.m. on March 23, 1952, the first infusion of the third course containing 20 mg. of pork ACTH in 750 ml. of 5% glucose solution was started. At 1:15 p.m. the patient suddenly developed severe pruritus over the upper extremities. Within the next half hour giant urticaria appeared over the arms, shoulders and upper trunk. There was itching of the scalp, intense angioneurotic edema of the eyelids and neck and wheezing respirations. The infusion was stopped, 200 ml. or 5.3 mg. of ACTH having been given. Twenty minutes later the itching had subsided. After another 30 minutes there was no wheezing and the angioneurotic edema was less severe. Three hours later the palpebral edema had disappeared. The temperature was 98.8°F. at noon, 101.0°F. at 3:30 p.m. and 99.6°F. at 8:00 p.m. Immediately prior to the infusion, the eosinophil count was 264 per cu.mm.; 30 minutes after the infusion it was 77 per cu.mm.; one hour later, 33 per cu.mm.; and two hours later, 11 per cu.mm. These observations suggest that in spite of the anaphylactoid reaction to ACTH sufficient hormone reached the adrenal cortex to effect a significant eosinopenia and possibly to suppress the reaction incited by itself. Subsequently, this patient was given sheep ACTH intravenously without reaction.

The fifth patient (JHH No. 259,036) was a 27-year old colored male with acute rheumatic fever. He was treated over a period of three weeks with small doses of pork ACTH given by slow intravenous infusion. The last five treatments were given with beef ACTH. His clinical response during treatment was satisfactory but 18 days later he had a recurrence and was again given beef ACTH by the intravenous route. Shortly after completion of the first infusion he had a chill, the temperature rose to 104°(R) and a maculo-papular eruption with generalized erythema appeared. Over the next week the temperature returned to normal levels and the eruption faded. He was then tested with 10 mg. of pork ACTH intramuscularly and had a similar reaction. Three days later the reaction was progressing in severity and he was given Compound F (Searle) 20 mg. by mouth every four hours for two days. The temperature was normal in eight hours and pruritus had diminished 40 minutes after the first dose. After 24 hours the eruption had faded and the eosinophil count had fallen from 352 to 44 per cu.

mm. Two days after the last dose of Compound F pruritus and erythema reappeared and the temperature rose to 102°(R). There was gradual improvement over the next three days.

DISCUSSION

These clinical observations demonstrate clearly that both ACTH and cortisone provide an effective form of treatment for the distressing manifestations associated with drug hypersensitivity reactions of the serum sickness type. Their use is particularly valuable in those patients who do not obtain prompt relief following the administration of epinephrine and the antihistamine drugs. A satisfactory response has been obtained with both ACTH and cortisone. However, the rapidity with which the pruritus and the objective abnormalities subside varies with the mode of administration. The most impressive response has been to the administration of cortisone orally. In most instances the initial dose has been 100 mg. followed by 50 mg. every two to six hours, depending on the severity of the sensitivity reaction and the clinical course of the patient. The preparation has the advantage of reasonable economy in addition to ease of administration. ACTH by intramuscular injection every six hours affords equally effective relief, but the problems of administration are more complicated for patient and physician. In the one case treated with a repository preparation of ACTH the rapidity of response was not quite as impressive as with cortisone given orally. The results with ACTH by intravenous infusion were also less rapid, but entirely satisfactory. This form of therapy is particularly useful if the patient must remain in the hospital and cannot take medication orally. Cortisone given intramuscularly even in large doses is unsatisfactory in this situation where a rapid effect is essential. In view of the fact that the responsible drug or foreign protein may be withdrawn, the treatment with ACTH or cortisone is necessary for only a short period of time. The relapses that have occurred after cessation of hormone administration have been relatively mild, of short duration, and in some instances responsive to antihistaminics.

During the past year a number of patients have been treated with ACTH by intravenous infusion. Great caution was exercised because of the danger of sensitivity and the development of a severe reaction to subsequent administration by this route. Although many patients have received prolonged treatment in this manner with repeated courses no serious acute reactions have been observed. Of the few reactions encountered all have been of the serum sickness type, and the incidence has been greater with the beef than with the porcine material.

Of interest is the blocking effect of ACTH and cortisone. In two patients who developed an allergic reaction, the one to penicillin and the other to PASA, cortisone and ACTH were able to suppress the clinical manifestations of the sensitivity reaction although administration of the offending drugs was continued. This procedure should be followed only when it is imperative to con-

tinue treatment with the drugs causing the allergic reaction, as, for example, in a case of subacute bacterial endocarditis where sensitivity to penicillin may develop and the disease cannot be controlled by any other antibiotic. There is no clinical evidence that ACTH or cortisone can prevent the possible production of periarteritis nodosa under such circumstances.

The results in the two cases of serum sickness with nervous system manifestations were interesting, but there is need for the accumulation of more experience with this type of involvement before any final conclusions can be drawn. Walsh (9) has recently reported a case of peripheral neuropathy and serous meningitis due to horse serum in which the administration of a total of 1.0 gm. of cortisone over a period of ten days produced no immediate or eventual benefit. It may be pointed out, however, that the failure of response in this case may not be significant as the dose administered was small. In these cases it would seem desirable to begin treatment as early as possible and to use large doses of either ACTH or cortisone.

SUMMARY

1. The use of ACTH, cortisone and Compound F-acetate in the treatment of 24 patients with a hypersensitivity reaction of the serum-sickness type is described. Thirteen of the reactions were due to penicillin, eight to horse serum, and one each to para-aminosalicylic acid, gold, and sulfonamides.

The response to therapy in the majority of the cases was prompt and complete. Satisfactory results were obtained with ACTH administered intramuscularly and by intravenous infusion, cortisone given orally and Compound F given orally and intramuscularly. The rapidity with which the various allergic reactions subsided varied with the mode of administration.

2. In two patients who developed an allergic reaction, one to penicillin and the other to para-aminosalicylic acid, cortisone and ACTH were able to suppress the clinical manifestations of the sensitivity reaction although administration of the allergen was continued.

3. Two patients with serum sickness accompanied by evidences of nervous system involvement were treated with ACTH intravenously. In both cases the neurological manifestations subsided with no residual disturbance of function. More experience is necessary before the significance of these results can be evaluated but in view of the prolonged dysfunction which may ensue early administration of large doses of ACTH or cortisone seems indicated in such cases.

4. Over 600 patients have been treated with ACTH in The Johns Hopkins Hospital, many reciving multiple courses of the hormone. Fifty received beef ACTH, seven sheep, and the remainder porcine hormone. In five patients an allergic reaction of the serum sickness type has been observed. In two cases the sensitivity was to porcine material, in two to beef, and in the other to hor-

mone derived from both sources. In one case the manifestations of the allergic reaction were suppressed during the administration of cortisone intramuscularly.

ACKNOWLEDGMENTS

Part of the cortisone and ACTH was purchased through a grant from the Division of Research Grants and Fellowships, National Institutes of Health, U. S. Public Health Service.

Part of the ACTH was supplied through the courtesy of Dr. Albert H. Holland of Armour and Company; part of the cortisone and the Compound F-acetate through the courtesy of Dr. James M. Carlisle of Merck and Company, and the Compound F through the courtesy of Dr. Irwin C. Winter of G. D. Searle and Company.

We wish to thank Drs. Warde B. Allan, Samuel P. Asper, Jr., Alan Bernstein, Walter Buck, John E. Howard and Charles W. Wainwright for permitting us to report cases under their care.

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HYPOKALIEMIA OCCURRING DURING PARA-AMINOSALICYLIC ACID THERAPY

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Received for publication October 13, 1952

Patients receiving para-aminosalicylic acid (PAS) were first noted to develop a low potassium syndrome by Cayley (1). To date, a total of 12 proved and 6 probable cases of hypokaliemia associated with PAS therapy have been reported (1–4). The discovery (5) that liquorice, a common flavoring vehicle for PAS, had a desoxycorticosterone-like action led two groups (3, 4) to assume that the liquorice, and not the PAS, caused the hypokaliemia. In 1951, an Australian group (6) published results of a study of the serum potassium in a large number of patients receiving a potassium salt of PAS, demonstrating that even patients on this form of PAS could develop a lowered serum potassium. This latter group concluded that, although liquorice acted as a contributory factor, impurities contained in the commercially available drug were responsible for the hypokaliemia (6, 7).

The observation that one of the patients receiving PAS on the Osler medical wards of The Johns Hopkins Hospital had two separate episodes of hypokaliemia early in the course of therapy with this drug stimulated interest in the possible explanation for this phenomenon. An effort was made to determine the incidence of hypokaliemia in a selected number of PAS treated patients, and common factors in such patients which might suggest the mechanism by which this drug produces a low potassium syndrome.

MATERIALS AND METHODS

The charts of 38 patients treated at The Johns Hopkins Hospital with PAS during the past one and one-half years were reviewed. None of these patients received liquorice. All received streptomycin or dihydrostreptomycin. The patients selected had been treated with PAS on the medical wards for no less than 4 weeks unless hypokaliemia developed earlier.

Because of the wide range of "normal" values for serum potassium, and because the electrocardiographic and clinical evidences for hypokaliemia do not always parallel the serum potassium level, patients were considered to have proved hypokaliemia only if at least one of the following criteria was present: 1) A serum potassium of 2.8 m.eq./l. or less, 2) A serum potassium between 2.8 and 3.0 m.eq./l. with either an electrocardiogram (EKG) showing changes compatible with hypokaliemia or the classical symptoms of hypokaliemia, 3) A serum potassium between 3.0 and 3.2 m.eq./l. with an EKG showing characteristic changes or 4) In the absence of a serum potassium determination, a record which included a characteristic EKG along with well described, typical symptoms of hypokaliemia.

EKG changes associated with hypokaliemia have been well described elsewhere (8–12). In this study the abnormalities considered significant were: 1) Prolongation of the Q-T interval, 2) Flattening or inversion of the T waves and 3) Sagging or depression of the S-T

segments. For an EKG to be considered definitive proof of hypokaliemia, it was required that one devoid of such changes be present either before or after the episode.

A multitude of symptoms occurring in association with proved hypokaliemia have been described (10, 11, 13–16). The most reliable symptoms and signs were considered to be weakness and flaccidity of limb musculature with depressed or absent deep tendon reflexes, in the absence of objective sensory changes.

INCIDENCE OF HYPOKALIEMIA IN PAS TREATED PATIENTS

On the basis of these criteria, these 38 patients can be divided into the following groups as shown in Table I.

1. Twenty-five patients showed no evidence of hypokaliemia during prolonged therapy with PAS.
2. Three patients had a complicated clinical course with symptoms suggesting hypokaliemia and were considered as "possible" hypokaliemia. The lack of serum potassium determinations, recorded EKG's and adequate descriptions of symptomatology makes more precise classification of these patients impossible.

TABLE I

Occurrence of Hypokaliemia in 38 Patients on Prolonged PAS Therapy

GROUP	NO. PATIENTS
No Evidence Hypokaliemia	25
Possible Hypokaliemia	3
Probable Hypokaliemia	2
Proved Hypokaliemia (Total of 12 episodes)	8

3. Two patients had prolonged episodes of well-described weakness, flaccidity and areflexia of limb musculature while on PAS. No EKG's or serum potassiums were obtained. These two patients are considered as "probable" hypokaliemia.
4. Eight of the patients were found, on the basis of the criteria mentioned above, to have had a total of twelve separate episodes of proved hypokaliemia (with a return of the serum potassium and EKG to normal, and disappearance of symptoms between episodes.) See Appendix for brief case reports of these patients.

ELECTROLYTE PATTERNS IN PATIENTS WITH PROVED HYPOKALIEMIA

Representative serum electrolyte determinations are presented in Table II. Omitting Patient # 10 because of her severe pre-existing renal disease, analysis of these electrolyte patterns in the patients with proved hypokaliemia in which appropriate determinations were made reveals the following:

1. In all, a pre-therapy hyponatremia and hypochloremia of varying magnitude was present. Pre-therapy serum carbon dioxide combining power and

potassium were within normal limits in all patients except #15 who consistently had a slight elevation of carbon dioxide. Pre-therapy serum electrolyte concentrations were therefore in accord with those reported by others (18–22) in patients with active tuberculosis.

2. With the exception of the terminal episode in Patient #1, during the periods of low potassium syndrome the serum carbon dioxide rose significantly and out of the range of normal in all patients in which it was determined. Coincidental drop in serum chloride was present in the majority.

TABLE II

Serum Electrolyte Patterns—Patients with proved Hypokaliemia

PATIENT	TIME[1] RELATED TO HYPOKALIEMIA	1ST EPISODE				2ND EPISODE				3RD EPISODE			
		CO_2	Cl	Na	K	CO_2	Cl	Na	K	CO_2	Cl	Na	K
		m.eq/l	*m.eq/l*	*m.eq/l*	*m.eq/l*	*m.eq/l*	*m.eq/l*	*m.eq/l*	*m.eq/l*	*m.eq/l*	*m.eq/l*	*m.eq/l*	*m.eq/l*
1[2]	Before	21.6	98.4	136.0	3.7	30.4	97.1	130.0	3.9	32.8	99.0	140.0	3.6
	During	28.4	92.0	127.0	3.2	34.7	93.5	138.0	3.0	31.3	91.0	136.0	3.0
	After	22.5	95.0	127.0	5.3	25.0	98.0	—	—	—	—		
15	Before	31.3	83.5	—	—	38.0	81.5	132.0	5.4			—	—
	During	>40	76.1	125.0	2.6	—	—	138.5	2.9				
	After	38.0	81.5	132.0	5.4	—	—	—	—				
18[3]	Before	29.2	79.2	—	—	33.4	95.4	141.0	3.8				
	During	34.3	77.3	134.5	2.8	—	89.2	137.0	3.2				
	After	29.5	89.1	131.0	4.4	—	—	—	—				
7	Before	27.6	88.6	—	—								
	During	—	—	—	2.7								
	After	23.3	95.9	131.0	4.6								
10	Before	19.9	102.0	—	—								
	During	16.6	75.0	113.5	2.7								
	After	23.3	88.0	121.0	4.3								
16	Before	27.6	91.9	123.0	4.1								
	During	38.8	85.2	128.0	2.8								
	After	29.2	90.1	137.0	4.6								
26	Before	—	98.4	132.0	5.2								
	During	—	—	—	—								
	After	—	—	146.0	4.5								
38	Before	25.8	85.0	—	—								
	During	—	93.0	135.0	2.9								
	After	29.2	94.0	130.0	4.0								

[1] In all patients except #18, chemistries, before first episode of hypokaliemia represent pre- PAS therapy determinations.

In all patients except #16, 18, 38, post-hypokaliemic chemistries were drawn within 8 days of the episode of hypokaliemia. In these exceptions the time interval was as great as four weeks.

[2] Receiving desoxycorticosterone 5 mgm. daily before and during third episode.

[3] Had received a total of 44 Gms. sodium PAS in the two days previous to first chemistries. Receiving desoxycorticosterone 5 mgm. and cortisone acetate 25 mgm. daily before and during second episode.

3. Several patients showed a slight increase in serum sodium during hypokaliemia. This is difficult to evaluate as in some of these it seemed merely a reflection of a tendency for a progressive elevation of an initially abnormally low sodium as clinical improvement in the original disease process occurred.

GASTRO-INTESTINAL INTOLERANCE TO PAS

It is well known that symptoms of gastro-intestinal irritation are frequently precipitated by the administration of PAS. In the first of these patients

followed by the author, severe vomiting and diarrhea were noted to be coincident with the episodes of hypokaliemia. Therefore, the incidence of gastro-intestinal intolerance to PAS in this series was determined, and the possibility of some correlation between such symptoms and the occurrence of hypokaliemia was considered. The frequency of vomiting and diarrhea in the 38 patients studied here, and the interrelationship between such symptoms and hypokaliemia is summarized in Table III.

In the group with proved hypokaliemia, there were 8 patients with a total of 12 separate episodes. Seven of these patients specifically denied any vomiting or diarrhea prior to admission. One patient, # 26, with tuberculous peritonitis had intermittent vomiting, but no diarrhea, for 2 weeks before admission. On PAS therapy, 6 of these patients had the onset of severe vomiting or diarrhea or both. Patient # 26 developed diarrhea for the first time. These gastro-intestinal symptoms were present in all 7 of these patients before and during their 11 episodes of hypokaliemia. The chart of the remaining patient

TABLE III

Incidence of Persistent Vomiting and Diarrhea and Relationship of Hypokaliemia to Such Symptom in Patients Receiving PAS

GROUP	NO. WITH PERSISTENT VOMITING & DIARRHEA	TOTAL NO. EACH GROUP
Proved Hypokaliemia	8 (?1)	8
Probable Hypokaliemia	2	2
Possible Hypokaliemia	3	3
No Evidence Hypokaliemia	1	25

(# 7) makes no mention of the presence or absence of these symptoms, but the stool chart shows a suggestive increase in the number of stools just previous to the time of hypokaliemia. In addition, the laboratory work sheet of this patient records 2 liquid stools, one just before and one during the episode of hypokaliemia. Among these patients, one spontaneously recovered from hypokaliemia when the dosage of PAS was decreased so as to alleviate the gastro-intestinal symptoms. Two others, who had early hypokaliemia, were able to continue on this drug without hypokaliemia after the subsidence of gastro-intestinal symptoms.

In the 2 patients with probable hypokaliemia, remission of the symptoms suggestive of hypokaliemia was coincident with the disappearance of the severe vomiting and diarrhea both had had. All 3 of the possible cases had severe diarrhea and intermittent severe vomiting.

Only 8 of the 25 patients with no evidence of hypokaliemia during prolonged therapy with PAS had any gastro-intestinal intolerance to the drug. In 7 of these 8, these symptoms were rapidly controlled by decreasing the dosage or discontinuing the drug. The remaining patient had severe, protracted vomiting

on many dosages and forms of PAS, but no diarrhea nor evidence of hypokaliemia.

DOSAGE AND TYPES OF PAS PRESCRIBED

It was not possible to establish a strict relationship between the dosage of PAS and the occurrence of hypokaliemia in this series. The dosage of this drug was widely varied in the group of patients with no evidence for hypokaliemia—one patient receiving as much as 25 gm. daily for 8 weeks without difficulty. In general, however, the patients who did develop hypokaliemia did so early in the course of therapy while receiving rather massive doses. Omitting Patient #16, a 50 pound adolescent, the average dosage and duration of therapy previous to the first episode of hypokaliemia was 24 gm. daily for 10 days.

Similarly, gastro-intestinal symptoms were not provoked by any single dosage level, but were in general associated with the administration of large amounts of PAS. In any one patient, the occurrence of these symptoms appeared to be a dose-related factor, in that a decrease in the daily intake of PAS generally alleviated such symptoms.

Although a number of different commercially available forms of PAS were used in treating these 38 patients, the majority received sodium PAS by mouth. In the 8 patients with proved hypokaliemia, 7 were on this form of the drug at the time of first onset of gastro-intestinal disturbances. Trial of intravenous sodium PAS and of a suspension of the acid form in several of these patients failed to relieve these gastro-intestinal symptoms, or to correct the hypokaliemia which appeared with or shortly after the vomiting and diarrhea. One patient (#26) developed diarrhea and hypokaliemia during therapy with only the intravenous form of sodium PAS.

The question as to whether impurities contained in commercially available PAS are responsible for the gastro-intestinal symptoms or hypokaliemia or both cannot be finally answered at this time as it was not possible to obtain samples of the PAS actually used before and during the episodes of hypokaliemia. Preliminary analyses of currently available PAS, however, show no impurity of the type described as the causative factor by the Australian group. Communication with the major supply house for the sodium PAS used in this series indicates that their product is virtually free of contamination (22).

DISCUSSION

It was of interest to try to ascertain the possible mechanisms for the production of hypokaliemia in tuberculous patients treated with PAS. Other authors have assumed that the occurrence of this syndrome of hypokaliemic alkalosis was in some way related to the therapy, and no consideration was given to the possible role of the primary disease process. Review of the liter-

ature reveals several reports on the electrolyte patterns in patients with tuberculosis (17–21). In 3 of these studies (17, 19, 20), the serum potassium was determined on a large number of patients with active pulmonary tuberculosis or tuberculous meningitis and the authors noted no significant alteration of the serum concentration of this electrolyte. Thorn (17) points out that there is no significant difference in the mean serum potassium levels in his group of patients with pulmonary tuberculosis, non-tuberculous pulmonary disease, and normal subjects. One is, however, struck by the unusually wide range of serum potassium concentrations (2.6–5.8 m.eq./l.) in his group of 92 patients with pulmonary tuberculosis as compared with his 2 control groups. Although no individual case reports are given, this suggests that perhaps alterations in potassium metabolism can accompany untreated tuberculosis. To the author's knowledge, only one case report of hypokaliemic alkalosis occurring in an untreated tuberculous patient has appeared in the literature (21). This patient, a child with tuberculous meningitis, had been on total intravenous alimentation for some time previous to the electrolyte studies reported. Hypokaliemia has been observed to occur in such patients associated with viomycin therapy (23).

The work of Harrison, Feinberg and Fleishman (20) on the electrolyte alterations occurring in a number of children with tuberculous meningitis suggests that the primary disease process is of considerable importance in the occurrence of hypokaliemia in tuberculous patients. This group found that, although the serum and red cell potassium concentrations were consistently within the normal range, these patients had a significant decrease in muscle potassium during the active phases of their disease. The clinical syndrome of hypokaliemic alkalosis was not observed in these children, some of whom were treated with streptomycin plus PAS, and some with streptomycin alone (24).

Concerning the present study, the lack of an adequate control series of untreated tuberculous patients, or non-tuberculous patients on PAS, is unfortunate. Moreover, in light of the above-mentioned studies and the fact that all of the patients in the present series were seriously ill, and many were severely undernourished previous to treatment, it is probable that they would prove to be unusually susceptible to any agent tending to produce hypokaliemia. Several factors, however, seem to incriminate PAS as the precipitating agent for the hypokaliemic episodes seen in these patients: the normal pre-therapy serum potassium levels in all patients in which it was determined, the rapidity with which the first episode of hypokaliemia followed the onset of PAS therapy, the spontaneous remission noted in a few upon withdrawal or decrease in dosage of the drug, and the later uneventful course of several. It should also be noted that the intake of potassium did not fall to such a low level as to be the sole causative factor in any of these patients. None were on total intravenous feedings and, although it is impossible to estimate their exact dietary

intake of potassium since many were anorexic, all were offered some type of standard hospital diet, the lower range of potassium content of these being approximately 70 m.eq. of potassium per day (5 gm. as KCl). For those patients completely unable to take food by mouth, tube feeding mixtures were provided which contained approximately 80 m.eq. of potassium per day.

One is left then to consider possible mechanisms by which PAS could produce excessive loss of potassium with a resultant hypokaliemic alkalosis. There are several ways in which this drug might cause increased renal excretion of potassium. Among these theoretical possibilities are:

1. Adrenal stimulation by, or adrenal hormone-like action of, PAS. Regarding this possibility, there are now several articles pointing out similarities between some actions of other salicylates and actions of ACTH and cortisone (25, 26). However, PAS has not been studied in this regard, and the present clinical material allows no judgment to be made on this possibility.

2. Alkalosis produced by excessive ingestion of sodium in the form of sodium PAS. As noted above, this was the major form used in this series. Twenty grams of the usual hydrated sodium salt of PAS contains 94.3 m.eq. of sodium. Trial of two other forms in 2 patients had no effect on the hypokaliemia. Although the excessive sodium may play a contributory role, the reported frequent decrease in serum potassium among patients receiving a potassium salt of PAS indicates that this is not the major etiological factor.

Since no balance studies were done on these patients, and none on similar patients has yet been reported, one cannot rule out some type of excessive renal loss. However, as was discussed above, the hypokaliemia occurring in these patients treated with PAS was apparently invariably associated with vomiting or diarrhea or both. On the basis of this evidence, it seems likely that these hypokaliemic episodes resulted primarily from loss of potassium through the gastro-intestinal tract secondary to vomiting and diarrhea produced by the PAS therapy. One cannot of course rule out other possible actions of this drug on the basis of the present material. Furthermore, the underlying deficit of muscle potassium, which appears to accompany active tuberculosis, and the increased excretion of potassium secondary to the increased ingestion of sodium are probably important contributory factors. But the occurrence of hypokaliemia in this series of patients on PAS seems to be too closely related to gastro-intestinal symptoms associated with the administration of the drug to be merely coincidental.

SUMMARY

Thirty-eight tuberculous patients treated with para-aminosalicylic acid were studied. Of these, 8 patients had definitely established hypokaliemia, 2 were considered as probably having hypokaliemic episodes, and 3 as possible hypokaliemia. Twenty-five patients showed no evidence of hypokaliemia.

Occurrence of hypokaliemia could not be related to the dose of PAS. All but one of the total of 13 patients with proved, probable and possible hypokaliemia had severe, persistent vomiting and diarrhea. The other patient in this group had suggestive evidence of diarrhea. In contrast, only one of the 25 patients with no evidence for hypokaliemia had severe and persistent gastro-intestinal disturbances on the drug. Although it is recognized that initial sub-clinical potassium deficit and the effect of increased ingestion of sodium contained in the sodium salt of PAS may well be important contributory factors, it is suggested that the hypokaliemia seen in these patients receiving PAS is in large measure secondary to gastro-intestinal disturbances associated with the administration of this drug.

ACKNOWLEDGEMENT

The author is grateful to Doctors J. E. Howard, S. Asper and C. G. Zubrod, as well as other members of the Departments of Medicine and Pharmacology of the Johns Hopkins University, for their advice and encouragement during this work.

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APPENDIX—CASE REPORTS

Case 1 (JHH 102387). A 42 year old colored female was admitted semi-comatose with miliary tuberculosis and tuberculous meningitis. Pre-therapy serum potassium and EKG were within normal limits. Throughout her course she was treated with PAS and intramuscular streptomycin (initially 3 gm. daily, decreasing to 1 g. daily by the 5th week of hospitalization), and for the first 13 weeks she received daily intrathecal injections of 100 mgm. of streptomycin. After 5 days of this treatment, during which time she received sodium PAS, 30 gm. daily by mouth, the serum potassium had decreased to 2.9 m.eq/l. Over the next 13 days the serum potassium remained low (range 2.8–3.2 m.eq./l.) and typical EKG changes of hypokaliemia were found to be present and persistent, despite changes from oral sodium PAS, first to a suspension of the acid form of PAS in lime water, and then to intravenous sodium PAS, 20 gm. daily. These variations failing to relieve either the hypokaliemia or the severe vomiting and diarrhea present from onset of therapy, PAS was temporarily discontinued, and the patient given 56 gm. of KCl by mouth over the next 3 days, with a prompt return of the serum potassium to normal, and a marked improvement in her sensorium and general status. Oral sodium PAS was again resumed (average daily dose of 15 gm.) with recurrence of persistent diarrhea and occasional vomiting. Shortly thereafter the serum potassium fell to the lower limits of normal, and remained so until the 8th week of treatment when vomiting became more frequent, and the patient was again found to have a serum potassium of 3.0 m.eq./l. and EKG changes compatible with hypokaliemia. Thereafter the patient was maintained on KCl, 6 gm. daily by mouth, and sodium PAS was increased to 20 gm. daily. This therapy was continued until the terminal period and was accompanied by vomiting and diarrhea of varying severity, but the serum potassium and EKG's remained normal over the next 14 weeks of treatment. In the 9th week, the intra-

thecal instillation of 100 mgm. of streptomycin, heretofore uneventful, was followed by a 12 hour period of unconsciousness with many transient neurological signs, but gradual complete recovery. In the 10th week, intrathecal injection of streptomycin was accompanied by a transiently weak, feeble pulse. Because of these episodes, bilateral frontal burr holes were made in the 11th week, and in the 13th week intrathecal injection of streptomycin was discontinued and intraventricular administration of this drug, 10–20 mgm. daily, was begun. After the 11th week, due to persistent hypotension, fasting hypoglycemia and lowered urinary 17-ketosteroids (0.42 mgm% per 24 hours), the patient was given 5 mgm. desoxycorticosterone intramuscularly daily. The intraventricular instillation of streptomycin was in general uneventful, except for transient episodes of confusion and occasional taps productive of a few drops of bright red blood. In the 22nd week of treatment, following such a traumatic tap, she became comatose and vomited frequently. Respirations were Cheyne-Stokes in character, and she displayed many transient neurological signs. On the day of death (some 36 hours after the above procedure) the serum potassium was again found to be 3.0 m.eq./l. A generalized convulsion was followed by complete flaccidity which persisted for the few hours previous to death. Autopsy showed massive intraventricular, intracerebral and subarachnoid hemorrhage. There was no evidence of active tuberculosis. There was some fibrosis of lungs and mediastinal nodes. Adrenals and other organs were essentially normal.

Case 7 (JHH 380244). A 67 year old colored male admitted because of empyema and broncho-pleural fistula of unknown etiology. He was placed on increasing doses of PAS to a maximum of 24 gm. daily. After 5 days of this therapy, he was noted to be confused and weak. Over the next 10 days he became frankly disoriented and had episodes of muscular tremor and fibrillations. On the 16th day of treatment, the serum potassium was 2.7 m.eq./l., calcium 8.1 mgm.% and phosphorus 2.5 mgm.%. An EKG showed abnormalities characteristic of hypokaliemia. After PAS was discontinued and 30 gm. of KCl was given in a 36 hour period by mouth, the serum potassium returned to normal, and the patient showed marked improvement in his sensorium and general condition. Subsequent course, without further PAS or KCl therapy, was uneventful.

Case 10 (JHH 119405). A 47 year old colored female with known pre-existing hypertension and nephritis was admitted with tuberculous peritonitis. Therapy with sodium PAS orally and per rectum to a total of 21 gm. daily was attended by severe vomiting. On the 15th day of therapy an EKG showed changes compatible with hypokaliemia. Four days later PAS was discontinued because of the severity of her gastro-intestinal symptoms. Over the next 3 days the patient continued to complain of severe weakness, and the serum potassium was then found to be 2.7 m.eq./l. The weakness gradually disappeared and the serum potassium rose to normal over the next week. This patient subsequently died in uremia, some 3 weeks after PAS was discontinued, with an abnormally elevated serum potassium. Autopsy revealed arteriosclerotic nephritis and a peculiar hyaline necrosis of enlarged retro-peritoneal nodes and of small tubercle-like masses scattered over the peritoneal surfaces. There was also marked diffuse scarring of the liver, fibrinous pericarditis and occasional submucosal hemorrhages in the intestine. Grossly the adrenals suggested some loss of cortical lipoid, but were normal microscopically.

Case 15 (JHH 501306). A 68 year old colored female was admitted with presumptive but unproven tuberculous meningitis. Therapy included the daily administration of streptomycin, 1–2 gm. intramuscularly and 100 mgm. intrathecally. After 15 days of widely varied doses of sodium PAS, orally and intravenously, this drug was discontinued entirely due to the severity of the vomiting and diarrhea it provoked. On the following day a serum potassium of 2.6 m.eq./l. and an EKG showing the changes of hypokaliemia were found. KCl, 6 gm. daily by mouth was started, and after 4 days, the serum potassium and EKG were

again normal. Sodium PAS, 20 gm. daily orally, was resumed and, after 2 weeks of this combined therapy, vomiting and diarrhea again became severe. Because of these latter symptoms, both drugs were discontinued for 1 week. Thereafter therapy with sodium PAS alone was resumed (16 gm. daily by mouth) and 4 days later the patient was found to be flaccid and areflexic. EKG at this time was again compatible with hypokaliemia, and the serum potassium was 2.9 m.eq./l. PAS was continued as above with an added 2 gm. of KCl by mouth daily. Vomiting became more severe, and the flaccidity and areflexia apparently did not abate. No further EKG's or serum potassiums were obtained. She died 8 days after the above findings, with terminal hypothermia (T: 94°F rectally) and respiratory distress. No autopsy was obtained.

Case 16 (JHH 364904). A 15 year old colored female with known disseminated tuberculosis since the age of 8 was admitted with tuberculous enteritis and peritonitis. Sodium PAS by mouth, 12 gm. daily, provoked severe vomiting and diarrhea. By the 8th day of therapy the serum potassium had decreased from an admission level of 4.1 m.eq./l. to 3.0 m.eq./l. An unsuccessful attempt to relieve the gastro-intestinal symptoms was made by changing to a suspension of the acid form of PAS. After a total duration of PAS therapy of 18 days the serum potassium was noted to be 2.8 m.eq./l. The acid form was then discontinued, and sodium PAS was resumed in doses of 6 gm. daily with relief of her vomiting and diarrhea, marked general improvement, and a spontaneous return of the serum potassium to normal.

Case 18 (JHH 564491). A 56 year old colored male was admitted with tuberculous meningitis and miliary tuberculosis. Therapy was immediately initiated with streptomycin, 2 gm. daily intramuscularly and 100 mgm. daily intrathecally, and with intravenous sodium PAS 20 gm. daily. On the third day of therapy his serum potassium was 3.2 m.eq./l. Sodium PAS was gradually increased to a daily maximum of 42 gm. orally and intravenously. On the 6th day of treatment, the serum potassium was 2.8 m.eq./l. and an EKG showed typical changes of hypokaliemia. After he was placed on maintenance doses of 4–6 gm. KCl by mouth daily, the serum potassium and EKG returned to normal and he became somewhat more responsive. Because of severe diarrhea, PAS was decreased to approximately 20 gm. daily, and was maintained at this level for the remainder of his course. The failure of the hyponatremia and hypochloremia present on admission to be corrected by variable amounts of NaCl. (maximum: 28 gm. daily) added to the diet suggested the possibility of adrenal insufficiency. Although eosinophile counts were repeatedly abnormally low (in the range of 22/cmm.) other tests of adrenal function, performed in the 6th–8th weeks of hospitalization, revealed: a flat response to an intravenous glucose tolerance test, a positive Mayo water test (night volume of 510 ml., largest day specimen of 300 ml. and an "A" value of 12), and lowered urinary 17-ketosteroids before and after a test dose of 25 mgm. ACTH (3.8 mgm. per 24 hours before and 2.7 mgm. per 24 hours after). Because of these findings, cortisone acetate, 25 mgm. daily intramuscularly, was started in the 9th week. In the 10th week, due to manometric and radiographic demonstration of a block in the flow of cerebrospinal fluid at the level of T3-4, bilateral parietal burr holes were made, and intrathecal streptomycin was decreased to 50 mgm. on alternate days, with the intraventricular administration of 50 mgm. of this drug on the other days. After the 19th week, desoxycorticosterone, 5 mgm. daily intramuscularly, was added to the cortisone therapy. In the 26th week the maintenance doses of KCl were decreased to 2–3 gm. daily. Serum potassiums and EKG's remained within normal limits until the 28th week when, following a 5 day period of unusual lethargy and severe diarrhea, the serum potassium was found to have decreased to 3.2 m.eq./l. and an EKG again showed a hypokaliemic pattern. On the day of these determinations he had a sudden episode of complete unresponsiveness and a marked fall in respirations and blood pressure. Although there was temporary improvement with stimulants and intra-

venous saline and glucose, he remained critically ill and died some 48 hours later. Autopsy showed no evidence of active tuberculosis. There was a left lateral intraventricular hemorrhage and a tuberculoma 2 cm. in diameter in the right occipital lobe of the brain. Adrenals were normal except for some decrease in cortical lipoid. The meninges were delicate except for dense adhesions and adherent dura and arachnoid at the level of T8.

Case 26 (JHH 598942). A 33 year old colored female was admitted with tuberculous peritonitis who had had some vomiting, but no diarrhea. Pre-therapy serum potassium was 5.2 m.eq./l. On the 3rd day of therapy with intravenous sodium PAS (20 gm. daily) the serum potassium was 4.3 m.eq./l. Over the next 48 hours the patient began to complain of severe weakness, vomiting and diarrhea. EKG obtained on the 4th day showed changes compatible with hypokaliemia. The serum potassium was not determined at this time. Variable amounts of KCl were given over the next 6 days, with subsidence of weakness and return of EKG to normal. Coincident with the diagnosis of hypokaliemia, intravenous sodium PAS, 20 gm. daily, was discontinued in favor of oral sodium PAS, 16 gm. daily. Diarrhea and vomiting abated over the next week, and the remainder of her course was uncomplicated by further gastro-intestinal symptoms or evidence of hypokaliemia despite several weeks of continued PAS therapy without added KCl.

Case 38 (JHH 354880). A 42 year old colored female was admitted because of tuberculous peritonitis. While receiving sodium PAS, 18 gm. daily by mouth, she developed severe diarrhea and vomiting. The serum potassium was found to be 2.9 m.eq./l. after 13 days of therapy. A trial of intravenous sodium PAS did not relieve the gastro-intestinal symptoms, and the serum potassium remained abnormally low (2.8–3.1 m.eq./l.). After a 10 day period of KCl therapy, 2 gm. daily, and oral sodium PAS, 12 gm. daily, she had subsidence of vomiting and diarrhea and the serum potassium returned to normal. Therapy with sodium PAS, 12 gm. daily by mouth, was continued over the next 7 weeks without further addition of KCl. Subsequent serum potassium levels and EKG's were normal, and the patient had no further gastro-intestinal symptoms.

ELECTRON MICROSCOPIC STUDIES OF THE ALTERATION OF COLLAGEN FIBRILS IN THE ARTHUS PHENOMENON

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Received for publication January 5, 1953

It is well known that marked alteration of collagen fibers (swelling, hyalinization, granularity, fragmentation, disintegration) is one of the effects of hypersensitive reactions of the anaphylactic type. Arthus, himself, in 1903, first described the striking swelling of the fibers at the site of the local anaphylactic reaction that bears his name (1); and the studies of Gerlach (2), twenty years later, redirected attention to this peculiar alteration of collagen in the local lesion (Arthus phenomenon) produced by the injection of the specific antigen into the tissues of the highly sensitized anaphylactic body. Focal swelling and degeneration of collagen fibers has become a subject of especial attention in recent years because of its prominent occurrence in the disseminated lesions of the important collagen-vascular diseases, among which are rheumatic fever, rheumatoid arthritis, periarteritis nodosa and disseminated lupus erythematosus. One of these disease states, periarteritis nodosa, has now been clearly shown to be producible by anaphylactic hypersensitivity, both experimentally and in man (3); and there are indications that hypersensitivity may play a role in the pathogenesis of other members of this group (3). It is of particular interest in this connection that when, under proper conditions, specific antigen finds its way into the blood stream and escapes into the tissues of the anaphylactically sensitized body, there occur disseminated foci of collagen swelling and degeneration, in appearance quite like the alteration of collagen fibers in the disseminated focal lesions of the collagen-vascular diseases. In man, disseminated collagen lesions of this character occur, for example, in the serum sickness type of protracted anaphylactic reaction resulting from hypersensitivity to drugs (3); and they have been produced experimentally in animals by Klinge (4), Rich and Gregory (5, 6) and others, using foreign serum as the sensitizing antigen. While swelling and degeneration of collagen fibers can occur at sites of injury produced by causes other than the meeting of anaphylactic antigen and antibody in the tissues, it is a particularly common and striking effect of anaphylactic hypersensitivity; and the fact that it is a basic characteristic of the serious collagen-vascular diseases renders it important to endeavor to learn all that one can regarding the nature, significance and pathogenesis of this peculiar type of connective tissue damage in each of the pathological conditions in which it occurs.

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The electron microscopic studies of Schmitt and his co-workers (7, 8), which have been confirmed by Vanamee and Porter (9) and others, have shown that even the smallest normal collagen fibers visible with the ordinary light microscope are composed of bundles of minute fibrils, each of which exhibits beautiful periodic cross striations when viewed with the electron microscope. Each period consists of two major bands,—a dense one which has been termed the A band, and a more transparent one designated as the B band. (Figs. 1 and 2.) While the spacing between bands of like density varies somewhat in different fibrils, and may be altered by certain procedures (e.g., stretching of the fibril), Schmitt and his co-workers (7) found that the most frequently occurring spacing is about 640 Å, which agrees with Baer's (10) x-ray diffraction studies of collagen structure; and they found the ratio of the lengths of the A and B bands to be fairly constant and equal to about 2.2. These general structural characteristics have been found in collagen fibrils obtained from different tissues of different animal species. Under certain conditions of study, additional transverse bands can be observed within the two major bands (8). Fixation and staining has been useful in bringing out these additional bands, but occasionally some of them have been visible in our unfixed, unstained preparations.

The present study was carried out in the attempt to determine whether alterations in the normal finer structure of collagen can be detected by the electron microscope in fibrils at the site of the reaction of the highly sensitized body to the local injection of the specific antigen (Arthus phenomenon).

EXPERIMENTS

Fourteen adult male albino rabbits were used in this study. Eight were sensitized by an intravenous dose of 10 cc. of sterile horse serum without preservative[2], per kilogram of body weight, and seven of these received, in addition, one to three subsequent intracutaneous injections of 0.1 to 0.4 cc. of the serum during two to six months following the intravenous injection. The remaining six animals were sensitized by two to four intracutaneous injections of 0.05 to 0.4 cc. of the serum at intervals during three months. The animals were given Purina Rabbit Chow Checkers and water *ad lib.*

Normal collagen was obtained from the skin and subcutaneous tissue before sensitization, and from normal sites in sensitized animals. When it was desired to study collagen at the site of an anaphylactic lesion, 0.15 to 0.4 cc. of horse serum was injected into the shaved skin of a sensitized animal, high on the flank near the back-bone, and the lesion was removed at the end of 16 to 48 hours after the injection, usually the latter. These lesions were erythematous and markedly edematous, with varying degrees of hemorrhage, and they ranged in size from 5 x 3½ cm. to 12 x 6 cm. They were typical Arthus phenomena of differing intensities. In several instances, in a given animal, collagen was obtained from one cutaneous site and again from another site at a later time, but in most instances the animals were killed with ether, or by an intravenous injection of air, before the removal of the site to be studied. In all cases, care was taken to avoid using for study a site that

[2] The horse serum was very kindly supplied by the Research Division of Sharp and Dohme.

had been subjected to an injection previous to the one given to produce the lesion that was to be studied.

In order to obtain clean fibrils of the minute size requisite for study with the electron microscope, a variety of procedures were tried. For example, thin strips of corium placed in normal saline in a Petri dish, were thoroughly teased apart with fine needles. After light centrifugalization to remove the coarser particles, the supernatant was centrifugalized at higher speed, the sediment of minute fibrils resuspended in fresh saline, and this repeated one or more times in order to cleanse the fibrils. Or, after moderate teasing in one or more changes of saline, the teased shreds were packed together upon the freezing microtome, cut at about 5 micra and the resulting particles transferred to normal saline in a small Petri dish and further reduced in size by agitation with a large needle. Or, after removing the muscle layer from a small rectangle of skin, the piece was placed directly upon the freezing microtome, epithelial surface down. Thin sections were cut and transferred to a small Petri dish of normal saline, in which they were broken up by teasing and agitation with a needle, and washed in one or more changes of saline, either with centrifugalization or simple gravity sedimentation.

Anyone who engages in the study of collagen fibrils with the electron microscope soon encounters the difficulty of obtaining clean fibrils, free from adherent material which obscures the periodic cross striations, and this difficulty is increased in the case of fibrils from the Arthus phenomenon, which are bathed in the protein-rich inflammatory exudate. Since collagen fibrils are resistant to the digestive action of trypsin, cleansing of the fibrils from adherent amorphous protein material can be greatly facilitated by exposing the fibrils to trypsin. In the present study, however, we wished to avoid the use of trypsin, for although under proper conditions it does not affect the structure of normal collagen fibrils (8, 9), it is quite possible that it might affect the altered collagen which is the object of our present study. Indeed, Sizer (11) reports that even mechanical fragmentation may increase the susceptibility of normal collagen to trypsin. The avoidance of the use of trypsin decidedly increases the difficulty of obtaining fibrils satisfactorily cleansed for electron microscopic study of structure.

In the electron microscopic study of the altered collagen fibrils at the site of the Arthus phenomenon, considerable difficulty was encountered in obtaining a sufficient number of altered fibrils in proportion to normal ones in each random drop of the suspension of fibrils. By no means do all of the fibrils at the site of an Arthus phenomenon undergo swelling and degeneration. The proportion of altered fibers and fibrils to normal ones varies in different lesions of this type; and in different parts of the same lesion the altered fibers and fibrils are distributed irregularly and focally among large numbers of normal ones. The focal distribution of altered fibers is readily visible with the light microscope in ordinary sections. In most sections of skin from the site of a cutaneous Arthus phenomenon, normal appearing collagen fibers greatly outnumber the swollen, deteriorated ones. When one considers this, and also the

small surface of the grid of the electron microscope, which accommodates only a single small drop of the saline suspension of fibrils, and also the circumstance that, for technical reasons, the fibrils should not be too closely massed upon the individual minute squares of the grid, it becomes obvious that the chance of obtaining a sufficient number of altered fibrils in a single, random drop of a suspension of fibrils that are taken at random from a lesion can be very small indeed; and this proved to be the case. After trying various procedures to increase the chance of obtaining a deposition of an adequate number of altered fibrils upon the grids, the following rather tedious but more effective method was adopted.

Immediately after killing the animal with ether or intravenous air, the lesion was removed and cut into six strips, transversely to the long axis; first, a strip from the site of the injection of the serum, one to two centimeters in dorso-ventral width; immediately below this a narrow strip, 3 mm. in width; then another broad strip, followed by a narrow one; and, finally, a third broad strip followed by a narrow one. The broad strips were immediately placed in normal saline in a covered Petri dish, and the corium of these strips was promptly used as the source of the collagen fibrils for electron microscopic study. The narrow strips were fixed in Zenker-formol, and paraffin sections were made and stained with hematoxylin and eosin for ordinary microscopic study of the character of the lesion at the different levels. All illustrations of fibers of light microscopic size are microphotographs of such preparations.

A full-thickness block, 0.5 x 0.5 cm. to 1 x 0.5 cm. in size, cut from the mid-portion of one of the broad strips, was placed, epithelial surface down, upon the freezing microtome and serial sections, about 10 micra in thickness, were made. As each individual section was cut, the tissue, whether coherent or fragmented, was removed from the knife, placed in normal saline upon a slide, covered with a coverslip and examined directly with the ordinary microscope. The first sections pass through the cutaneous muscle layer immediately below and closely bound to the corium. All sections containing any muscle fibers were discarded, and the sectioning was continued into the lower layer of the corium, each successive section being examined microscopically before cutting the next one. The individual normal collagen fibers of the corium are, for the most part, really bundles of parallel, smaller refractile fibers, which give the unstained fiber-bundle the appearance of having longitudinal striations. In this unfixed, unstained material altered fibers are recognizable by the diminution in refractility. The alteration may affect only a segment of a fiber-bundle, and the affected part is commonly swollen, loses its normal longitudinal "striations", and the small, refractile, normal fibers of the bundle become fused into a homogeneous, less refractile, hyaline mass. We have never encountered this alteration in preparations of fibers from normal corium. When a frozen section was found to contain numerous altered fibers, the microtome was set to cut at about 3 micra, and the fragmented material of about 20 successive sections was transferred from the knife to 4 or 5 cc. of normal saline in a little dish. Further dispersion of the fibrils was achieved by agitation with a large needle, and this was continued for about five minutes to facilitate washing of the fibers. A number of samples of the suspension were then examined with the ordinary microscope, and if numerous altered fibers were seen, grids for the electron microscope were prepared after brief gravity sedimentation; or, following a longer sedimentation, about two-thirds of the supernatant was carefully withdrawn and replaced by fresh saline, the fibers were washed

by further agitation with the needle, and the suspension re-examined. If an inadequate number of altered fibers was found in the suspension, the entire procedure was repeated, using another strip of the lesion.

In the preparation of each grid, a single drop of the fibril suspension was transferred by means of a capillary pipette to a grid coated with 2 per cent collodion, care being taken to prevent overflow of the drop beyond the margins of the grid. This ensured the deposit of all of the fibrils in a full drop of the suspension. The drop was allowed to dry about three inches below a 50 watt electric light bulb. The grid was then freed from salt crystals by gentle immersion in distilled water, again dried, shadowed with chromium, and examined under the electron microscope. 55 electron micrographs of fibrils from normal corium were made, and 145 electron micrographs of fibrils from corium at the site of Arthus phenomenon lesions.

The major two-band periodicity of *normal* collagen fibrils, as illustrated in Figs. 1 and 2, conforms to that previously observed by others (7, 8, 9). In some of our preparations, as mentioned above, a narrow additional band appears in one of the major bands. Bands in this location, and also in the other major band of the period, have been previously observed (8).

In the study of the material from the *anaphylactic lesions*, it became clear from direct observation with the electron microscope, and from the micrographs of the specimens, that even with the precautions that were taken to obtain the maximum number of altered fibrils, most of the fibrils deposited upon the grids were normal ones. This, while increasing the difficulty of the study, was not surprising for, as was pointed out above, most of the collagen fibers of the corium in ordinary stained sections of the cutaneous Arthus phenomenon are normal in appearance, and samples of the unfixed, unstained fiber suspensions used for the electron microscopic study showed that most of the fibers of a size visible with the light microscope were normal ones. However, in 16 (11%) of the 145 electron micrographs of collagen fibrils from Arthus phenomenon lesions, there were 51 examples of fibrils which we interpret as specifically altered ones. The cross-bands in these fibrils are either completely obliterated (Figs. 7, 9, 11), or there remain only irregular suggestions of remnants of bands (Fig. 5, at X), or at most a few vague bands, the spacing of which does not correspond with that of the bands in the periods of adjacent normal fibrils (Fig. 6, at D). These altered fibrils have, for the most part, a smooth, hyaline appearance, but irregularly distributed differences in density in different regions of a given fibril are often evident (Figs. 5, 6, 7, 9, 11). The lateral margins of the altered fibrils may be straight and smooth, but often they are irregular, having a rather "nibbled" or ragged appearance (Figs. 5, 6, 7, 9). The ends of some of these fibrils have an appearance suggesting that the fibril had been fractured (Fig. 9, at X). While the ordinary light microscope shows that altered collagen fibers in anaphylactic lesions fracture readily (Figs. 15, 16, 18), normal collagen fibrils are highly resistant to fracturing. We have encountered only one fractured normally

cross-striated fibril among the great number of normal ones in our 200 electron micrographs of fibrils which have been subjected to teasing and beating with needles.

As regards swelling of the altered fibrils, it cannot be stated with certainty that a particular altered fibril or fibril-bundle represents a swollen one, for its width in its previously normal state is, of course, not known. However, the large size of some of these altered fibrils, in comparison with the size of the normally striated fibrils in these preparations, suggests very strongly that they have undergone considerable swelling. Compare, for instance, Fig. 9 with the fibrils and fibril-bundles in Fig. 3, which is of the same magnification (×14,360). Also, note that the hyaline fiber in the lower part of Fig. 11 shows a distinct enlargement at A, and compare this with the similar enlargement in the swollen fiber visible with the ordinary microscope in Fig. 18.

That fibrils of this type, visible with the electron microscope, represent anaphylactically altered collagen fibrils is supported by the following circumstances: (1) No fibrils with identical characteristics were found in any of the 55 electron micrographs of collagen from normal skin, in which hundreds of normal fibrils are pictured. (2) In the same electron microscopic field, these altered fibrils are found in sharp focus among perfectly normal fibrils that have the normal periodic structure (Figs. 5, 6, 7). (3) The electron microscopic characteristics of these fibrils, apart from the status of the finer, periodic structure which, of course, cannot be seen with the ordinary light microscope, are quite similar to those of the anaphylactically altered larger fibers visible with the ordinary microscope. Thus, as in the case of these minute altered fibrils, the larger, anaphylactically altered ones visible with the ordinary microscope also commonly have a hyaline appearance, often with irregularly distributed differences in density (Figs. 8, 10, 15, 19, 20); the margins of these larger altered fibers, also, are often irregular and ragged (Figs. 8, 16, 17, 18, 19, 20); their ends, too, often have the appearance of having been broken and, indeed, in stained sections one can readily see that the swollen, hyaline collagen fibers at the site of the Arthus phenomenon fracture readily (Figs. 15, 16, 18).

Swelling, hyalinization, variations in density, ragged margins and fragmentation are likewise familiar characteristics of the altered collagen fibers in ordinary microscopic sections of the lesions of the collagen-vascular diseases (Fig. 21).

DISCUSSION

As was stated above, the smallest individual collagen fibers visible with the ordinary light microscope are composed of bundles of minute, cross-banded fibrils that are visible with the electron microscope but are too small to be seen with the ordinary microscope. The bundles of minute fibrils which consti-

tute the fiber visible with the ordinary microscope are infiltrated with and surrounded by amorphous ground substance. Gross (14) correctly points out that it is not possible to be certain, from examination with the ordinary microscope, whether pathological alterations in the fibers observed in histological sections represent alterations in the minute fibrils of which the fibers are composed, or alterations in the amorphous substance between and covering those fibrils. Clarification of this important point can be obtained only by a study of the minute fibrils, themselves. While the periodic bands of normal fibrils may be obscured by adherent amorphous material, and while the limits of technical artefacts in electron microscope preparations are not yet completely defined (artefacts may suggest, somewhat, some of the abnormalities described here), we have not seen, in our electron microscope preparations of normal collagen, fibril abnormalities that actually conform to those that we have described and pictured here in the electron micrographs of fibrils from the Arthus phenomenon. These fibril abnormalities are interpreted as representing a pathological alteration, and one that is strikingly similar in appearance to that visible with the ordinary microscope in the collagen fibers at the site of the Arthus phenomenon. If this interpretation is correct, it is of importance in indicating that the local anaphylactic reaction produces an alteration of the elementary collagen fibrils themselves, and not simply an alteration of ground substance in and about the fibril bundles. It is, of course, possible that the fibril alteration may be preceded by, and may conceivably result from, a primary alteration of the ground substance. It is well known that collagen will disintegrate in acid solutions (9), and it is possible that fibril alterations of the type described here may be the results of focal acidity in the inflamed area.

Some of the electron micrographs of altered fibrils pictured in the illustrations of the present paper were sent to Dr. Jerome Gross of the Massachusetts General Hospital for examination, in order to learn whether he had ever encountered fibrils of this nature in his very extensive electron microscopic studies of normal collagen over a period of years. We have his permission to say that he has not encountered such fibrils in his studies. He expressed the opinion that they "may very well be swollen collagen," but that definite proof of this would require the demonstration of a transition from normal collagen structure to the abnormal in the same fibril. We are in agreement with that opinion, and we had, of course, sought for such transitions; but so far we have not obtained electron micrographs of fibrils showing transitions of that sort. The closest approximation to a demonstration that these altered fibrils formerly had a cross-banded structure is represented in Figure 6, in which irregularly spaced remains of cross-banding are visible at D.

The question arises as to why the swollen fibrils have an increased density. Since the nature of the alteration responsible for the swelling of the fibers

in the Arthus phenomenon is not known, that question cannot be answered at present. It can be said, however, that the swollen fibers, of a size visible with the light microscope, in the unstained, fragmented frozen sections from which the electron microscopic preparations were made, and also the swollen fibers in unstained paraffin sections of the same lesions, appear less refractile and more dense than the normal collagen fibers. It would appear that the swelling and hyalinization of the fibers represents some alteration other than the mere imbibition of transparent fluid.

The difficulties in obtaining from the Arthus phenomenon an abundant yield of altered fibrils in a condition suitable for electron microscopic study were outlined above, and these difficulties have left us with fewer satisfactory electron micrographs of altered fibrils than we would desire. We are continuing the study, with the particular purposes of attempting to determine definitely the character of the altered fibrils, to detect the nature of the early changes in the bands of the periods before they disappear, and to compare the alteration in the finer structure of the fibrils at the site of the anaphylactic Arthus phenomenon with that in the lesions of the collagen-vascular diseases. Meanwhile, we have considered it desirable to report the present observations, for, with the exception of Wolpers (16), others have failed to find, with the electron microscope, any abnormal fibrils in the lesions of the collagen-vascular diseases, even though, as is well known, the ordinary light microscope reveals in those lesions alterations in the collagen fibers which are indistinguishable from those produced by anaphylactic hypersensitivity. Thus, Kellgren and his coworkers (12), in an electron microscopic study of material from the subcutaneous nodules of rheumatoid arthritis, found only normal fibrils with normal band periodicity, or amorphous debris; this was the experience of Gross (15) in a similar electron microscopic study of material from rheumatoid nodules and from the skin of patients with disseminated lupus erythematosus and scleroderma; and Gale (13), in an electron microscopic study of material from the lesions of rheumatoid arthritis, rheumatic fever and disseminated lupus erythematosus, found "no significant change in structural characteristics and no change in periodicity of the fibrils". In none of these electron microscopic studies were fibrils with altered structure found. In an electron microscopic examination of collagen from the necrotic portions of rheumatic nodules that had been present for two years, Wolpers (16) encountered fibrils with no cross striations. In the non-necrotic portions of the lesions, and in collagen from the Arthus phenomenon, the fibrils had normal cross striations. Wolpers concluded that even in areas in which the so-called "fibrinoid alteration" is visible with the light microscope, the finer structure of the fibrils remains intact, and that it is not the collagen fibril but the ground substance which is altered in the Arthus phenomenon and in the rheumatic lesions. He regarded the loss of striations in some of the fibrils in the necrotic areas as

secondary to the necrosis. It is doubtless true that collagen fibrils degenerate in areas of necrosis, but since even in non-necrotic foci in the Arthus phenomenon and in the lesions of the collagen-vascular diseases individual collagen fibers of a size visible with the ordinary light microscope exhibit swelling, hyalinization, variations in density, irregular edges and fragmentation,—alterations which may progress to the conversion of the fibers into amorphous debris, it seems improbable that the smaller fibrils of electron microscopic size, of which the larger fibers are composed, remain normal. It would seem more likely that the failure to discover altered fibrils in non-necrotic portions of these lesions in the above-mentioned electron microscopic studies may have been due to the difficulties that we have mentioned, and to the fact that in older lesions many of the altered fibers disintegrate into amorphous debris. The finding of altered fibrils of electron microscopic size in the present study of anaphylactic lesions, warrants not only the investigation of the manner in which the fibril periodicity becomes lost in these lesions, but also further electron microscopic study of the fibrils in the lesions of the collagen-vascular diseases.

SUMMARY

Electron microscopic study of collagen fibrils obtained from local anaphylactic lesions of the Arthus phenomenon type has revealed fibrils which exhibit absence of the normal periodic cross-striations, hyaline transformation, variations in density, irregularities of the margins and evidences of swelling and fragmentation. Further studies are being carried out with the purpose of determining the nature of this alteration, and of comparing the alterations in the finer structure of the fibrils in the lesions of the collagen-vascular diseases with those in experimentally produced local anaphylactic lesions.

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LEGENDS FOR ILLUSTRATIONS

FIG. 1. Electron micrograph of normal collagen fibrils from skin of rabbit (1187C), showing the characteristic periodic cross-bands. Magnification, ×43,600.

FIG. 2. Electron micrograph of normal collagen fibrils from skin of rabbit (1187E), showing the characteristic periodic cross-bands. Magnification, ×43,600.

FIGS. 3 and 4. Same micrographs as Figs. 2 and 1, but at magnification of only 14,360, for comparison with the fibrils in Figs. 5, 6, 7, 9 and 11 which are also magnified 14,360 times.

FIG. 5. Electron micrograph of collagen fibrils from Arthus phenomenon in skin of rabbit (1366A). At X, several irregular dark lines suggesting remains of cross-bands can be seen in the curved, hyaline altered fibril. Compare this fibril with the altered fibrils of a size visible with the ordinary light microscope in Fig. 8. Y is a normal fibril. Magnification, ×14,360.

FIG. 6. Electron micrograph of collagen fibrils from Arthus phenomenon in skin of rabbit (1367B). A, B, C, and D are altered fibrils. Irregularly spaced remains of cross-bands at D. Compare these fibrils with the altered fibrils of a size visible with the ordinary light microscope in Figs. 8, 16, 17 and 18. Normal fibrils at E and F. Magnification, ×14,360.

FIG. 7. Electron micrograph of collagen fibrils from Arthus phenomenon in skin of rabbit (1367C). At X, an altered, hyaline fibril with loss of cross-bands and ragged margins. Compare this fibril with the altered fibrils of a size visible with the ordinary microscope in Figs. 8, 16, 17, 18. At Y, a normal fibril. Magnification, ×14,360.

FIG. 8. Ordinary light microscope photograph of swollen, hyaline collagen fiber from Arthus phenomenon in skin of rabbit (305) H and E sections. Note the ragged margins of part of the fiber. Magnification, ×615. This altered fiber and those in the other ordinary light microscope photographs of Figs. 10, 15, 16, 17, 18, 19 and 20 are relatively enormous in comparison with the fibrils in the electron micrographs. Since these fibers are magnified only 615 times, the pictures would have to be enlarged twenty-three times to provide a proper size comparison with the electron micrographs of Figs. 5, 6, 7, 9 and 11, which represent a ×14,360 magnification.

FIG. 9. Electron micrograph of branching, altered collagen fibrils from Arthus phenomenon in skin of rabbit (1365B). Note loss of cross-bands, hyaline appearance, irregular margins of upper and lower branches, and fractured appearance of end (X) of middle branch. Magnification, ×14,360. Note similarity to the altered, branching fibers of a size visible with the ordinary light microscope in Fig. 10.

FIG. 10. Ordinary light microscope photograph of branching, hyaline, collagen fibers from Arthus phenomenon in skin of rabbit (543). Magnification, ×615. Note similarities to Fig. 9, including the localized, knob-like irregularity to which the arrow in each picture points. See comment regarding size in legend of Fig. 8.

FIG. 11. Electron micrograph of altered collagen fibrils from Arthus phenomenon in skin of rabbit (1362D). Note hyaline appearance, enlargement of branch at A, and irregular densities suggestive of altered remains of cross-bands in lower right branch. Magnification, ×14,360. Compare with Fig. 18.

FIG. 12. (*This and all following illustrations are ordinary light microscope photographs.*) Normal collagen fibers, separated by edema, at extreme edge of Arthus phenomenon in skin of rabbit (314). Magnification, ×74. Compare the widths of these fibers with those of the fibers in Fig. 13.

FIG. 13. Enormously swollen collagen fibers, widely separated by edema, in Arthus phenomenon in skin of rabbit (539). Same magnification as the normal fibers in Fig. 12 (×74), and at same level of the corium.

FIG. 14. Normal collagen fibers in skin of rabbit (301) at magnification ×615. Compare widths of the fibers with those of the swollen fibers at same magnification in Figs. 15, 16, 17, 18, 19 and 20.

FIG. 15. Swollen, hyaline, collagen fibers in Arthus phenomenon in skin of rabbit (308). Magnification, ×615. Note irregular density, and fractures marked X. The black spots in this and in the following pictures are leucocytes of the inflammatory reaction.

FIG. 16. Swollen, hyaline, collagen fibers in Arthus phenomenon in skin of rabbit (314). Magnification, ×615. Note ragged margins and fractures.

FIG. 17. Swollen, hyaline, collagen fiber in Arthus phenomenon in skin of rabbit (314). Magnification, ×615. Note irregular margins.

FIG. 18. Swollen, hyaline, collagen fibers in Arthus phenomenon in skin of rabbit (539). Magnification, ×615. Note fracture, ragged margins of fiber X, and enlargement of branch Y similar to branch A in electron micrograph of Fig. 11.

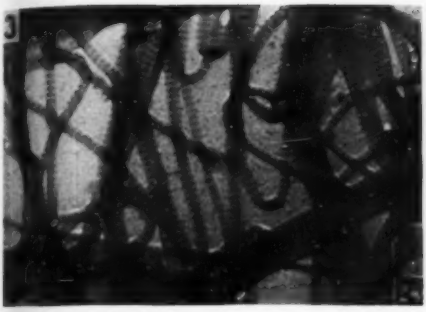
FIG. 19. Swollen, hyaline, collagen fiber in Arthus phenomenon in skin of rabbit (308). Note irregularities in density and ragged margins. Magnification, ×615. Compare with Fig. 21.

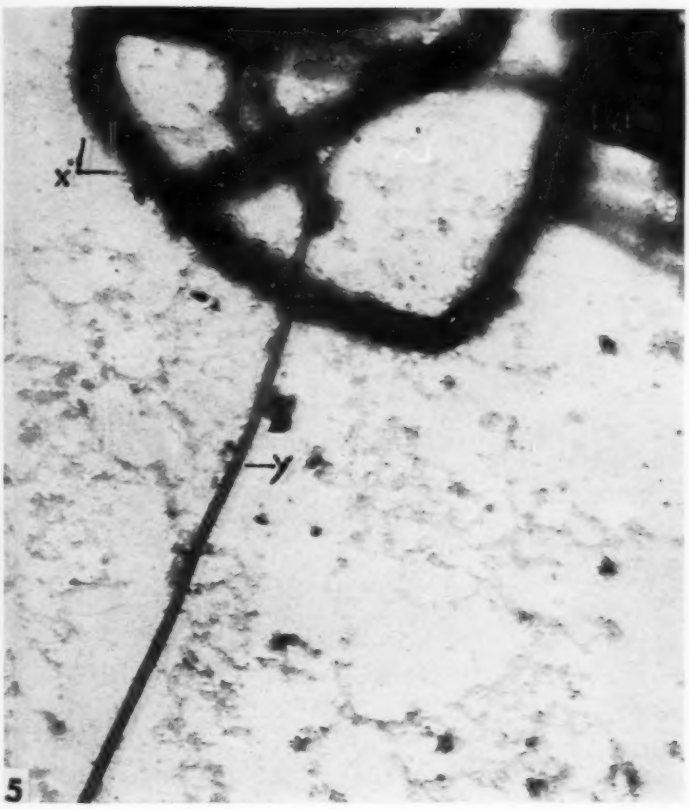
FIG. 20. Swollen, hyaline, branching collagen fibers in Arthus phenomenon in skin of rabbit (301). Note irregularities in density and ragged margins. The black spots are inflammatory cells. Magnification, ×615. Compare with Fig. 21.

FIG. 21. Swollen, hyaline, branching collagen fibers with irregularities in density and ragged margins, in subcutaneous nodule from a case of acute rheumatic fever (S.P. 91381). Compare with the altered fibers in the Arthus phenomenon in Figs. 19 and 20. Magnification, ×615.

FIG. 1

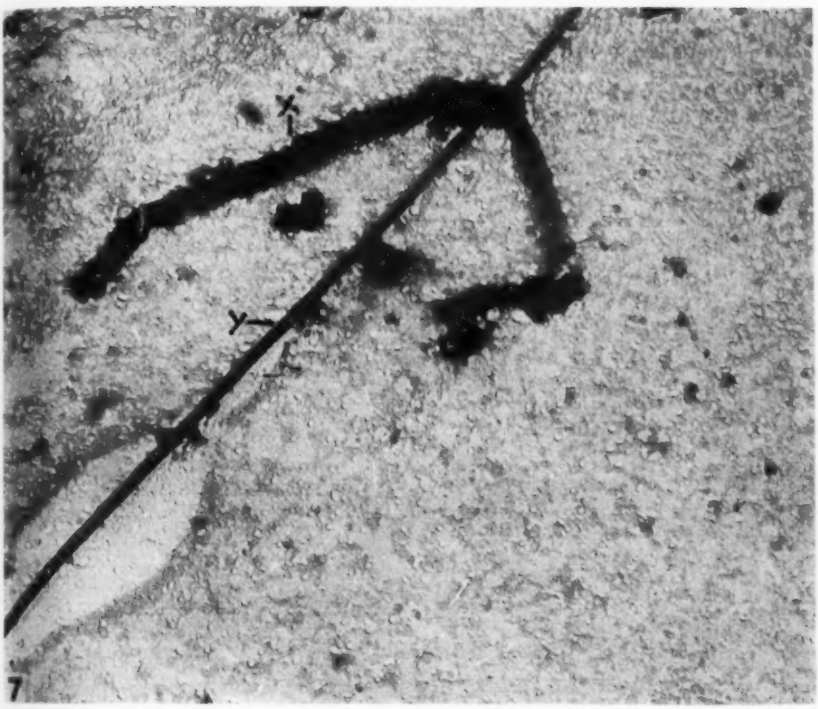
FIG. 2

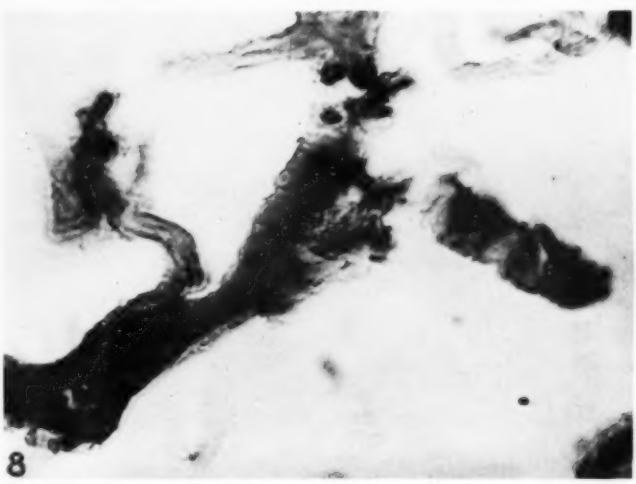




FIGS. 4–5

FIG. 6





FIGS. 7–8

FIGS. 9–10

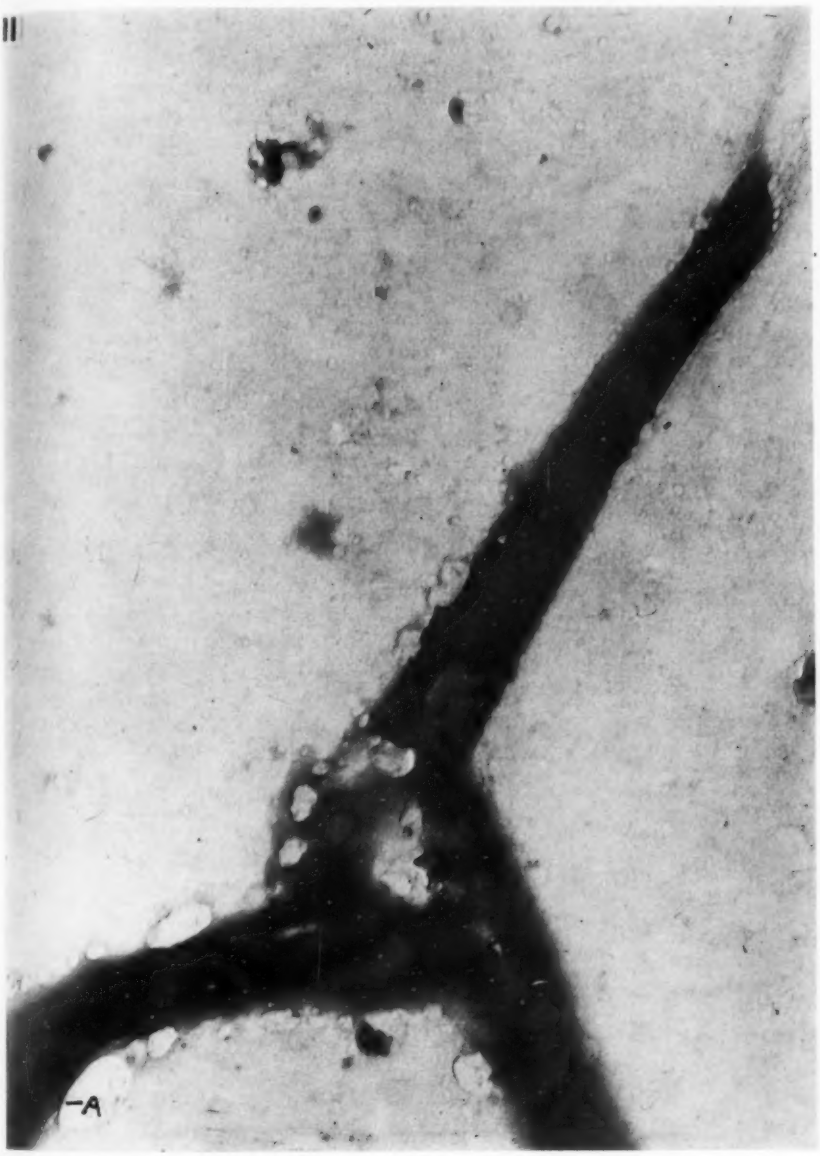
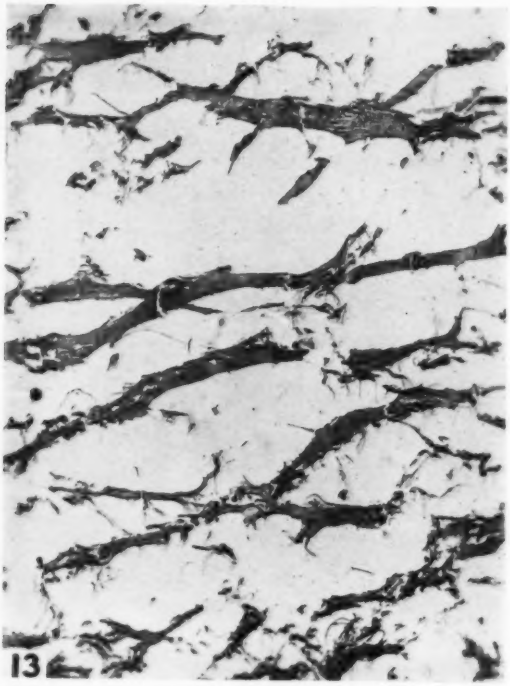
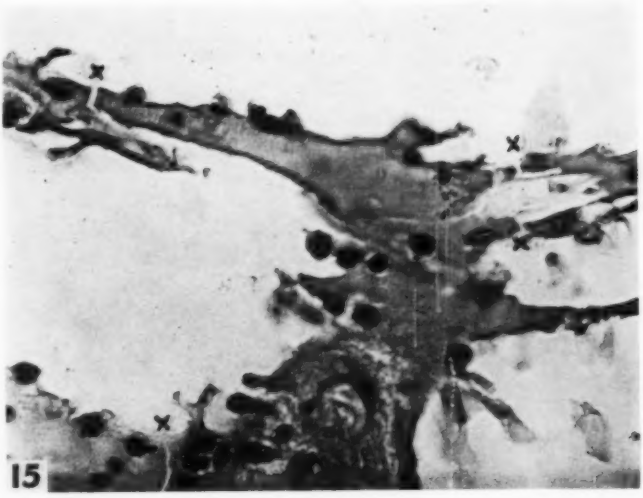


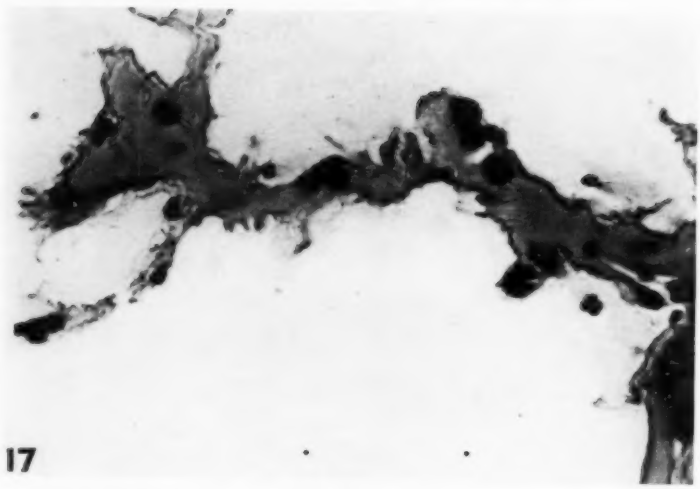
FIG. 11

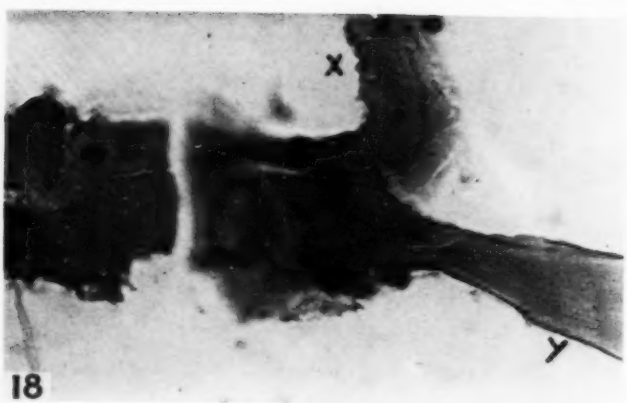


FIGS. 12–13

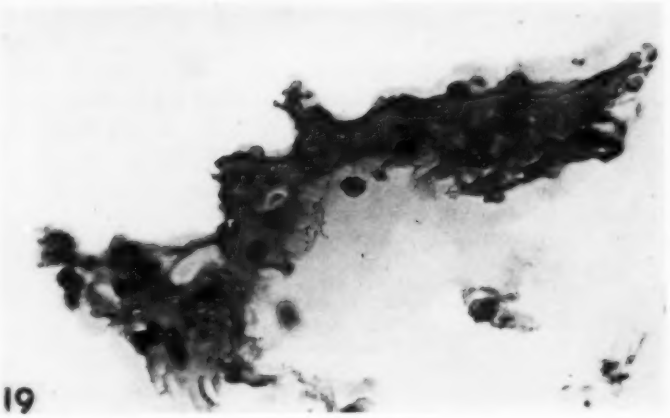


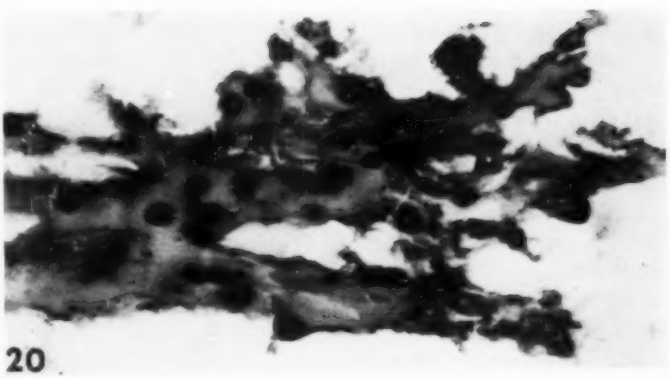
FIGS. 14–15





FIGS. 16–18





FIGS. 19–21

THE DETECTION OF PREGNANDIOL AND PREGNANTRIOL IN THE URINE OF PATIENTS WITH ADRENAL HYPERPLASIA. SUPPRESSION WITH CORTISONE

A Preliminary Report*

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Received for publication January 26, 1953

Butler and Marrian (1) have described the presence of 3-α-17-α-20-pregnantriol (hereafter referred to as pregnantriol) in the urine of two women with the adrenogenital syndrome. The clinical details are not clear and this steroid disappeared from the urine of one subject after unilateral adrenalectomy. Mason and Kepler (2) subsequently isolated the same compound from the urines of three women with pseudohermaphroditism and adrenal hyperplasia, and from a single patient with an adrenal tumor, although the latter diagnosis was not confirmed by exploration. Miller and Dorfman (3) also discovered this compound in significant quantity in the urine of a "young woman with masculinization." Although the clinical data are brief in these reports, undoubtedly some of the patients had the adrenogenital syndrome due to adrenocortical hyperplasia as defined by Wilkins (4).

The occurrence of increased quantities of 3-α-20-pregnandiol (hereafter referred to as pregnandiol) in the urine of individuals with the adrenogenital syndrome has been reported by Venning *et al.* (5), and Genitis and Bronstein (6) in a total of four patients. The clinical description in the former report is not detailed and in one instance an adrenal tumor was present. Stover and Pratt (7) reported no pregnandiol in a single case.

Pregnandiol is known to be a metabolite of progesterone, occurring in moderate amounts in the urine of normal adult females, increasing the second half of the menstrual cycle and in pregnancy. Mason (2) states that by analogy pregnantriol may represent a metabolite of 17-hydroxyprogesterone.

The purpose of this preliminary report is to show that pregnantriol as well as increased quantities of pregnandiol occur regularly in the urine of patients with the adrenogenital syndrome due to bilateral adrenal hyperplasia and that both are suppressed with cortisone. A relatively simple screening procedure to detect the presence of pregnantriol will be described and evidence will

* This work was made possible by a Grant-in-Aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council, and by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, United States Public Health Service.

be offered that the method of Sommerville and Marrian (8) for the measurement of pregnandiol may be influenced by the presence of pregnantriol.

MATERIALS AND METHODS

Specimens of urine were preserved with 25 per cent acetic acid and stored in the cold room at 4°C. Subjects included normal adults and children and young patients with clinical and laboratory evidence of the adrenogenital syndrome due to adrenocortical hyperplasia (4). One patient with a virilizing adrenocortical tumor and two with Cushing's syndrome without tumor are included.

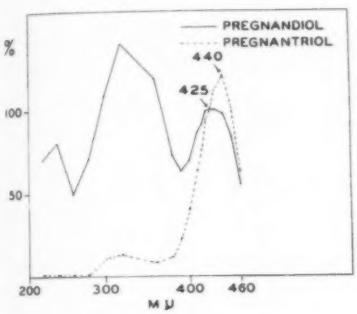

FIG. 1

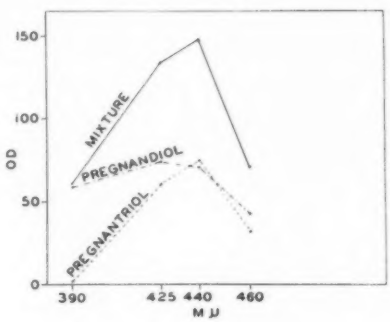

FIG. 2

FIG. 1. A comparison of 3-α-17,20-pregnantriol and 3-α-17-pregnandiol in concentrated sulfuric acid through the ultraviolet and visible spectrum. These are plotted as percentage absorption with reference to the absorption of each compound at 425 mμ, in order to accentuate the differences at 440 mμ and below 400 mμ.

FIG. 2. Optical densities of pregnandiol (75 gamma) and pregnantriol (50 gamma) in concentrated sulfuric acid at 390, 425, 440 and 460 mμ. The addition of 50 gamma of pregnantriol to 75 gamma of pregnandiol results in a peak at 440 with relatively less absorption below 400 mμ as compared to pure pregnandiol. With the combination, the optical density at 425 mμ (commonly employed for the measurement of pregnandiol) appears to be a summation of the two compounds at this wavelength.

A sample of pure pregnantriol, kindly supplied by Doctor Harold Mason, was compared with pregnandiol through a broad range of the visible and ultra-violet spectrum (Figure 1). A marked difference in the absorption spectra by the two compounds was observed below 400 mμ. In addition while pregnandiol showed a peak at 425 mμ (the point ordinarily employed in quantitative measurements of this compound) with diminishing absorption above this point, pregnantriol demonstrates greater absorption at 440 than at 425 mμ. The addition of 50 gamma of pregnantriol to 75 gamma of pregnandiol introduces a peak at 440 mμ (Figure 2). Formulae have been derived in an attempt to determine the amount of each compound present in mixtures and their validity is presently being tested.

The method of Sommerville and Marrian (8) was employed for the measurement of pregnandiol in the urine. The compounds were extracted with toluene by refluxing and rigidly purified according to the described technique. The final residues were dissolved in concentrated sulfuric acid and the resultant chromogens read with the Beckman DU quartz spec-

trophotometer. In order to express the quantities as pregnandiol (Table I), a correction factor was applied according to the principles set forth by Allen (9) in order to minimize the effect of non-specific chromogens.

$$\text{corrected OD} = \text{OD } 425 - \frac{\text{OD } 390 + \text{OD } 460}{2}$$

Application of this formula to various quantities of pure pregnandiol resulted in a straight line relationship between the corrected optical density and the amount of the compound.

TABLE I

PATIENT	SEX	AGE	DIAGNOSIS	*PREGNANDIOL (MG. PER 24 HRS. URINE)	
				Before Rx	with Cortisone Rx
AB	M	31	Normal	0.1-0.29	
HS	M	33	Normal	0.15	
MT	F	29	Normal		
			(early cycle)	0.4	
			(late cycle)	15.0	
JA	M	4 yr.	Normal	0.05	
MC	F	4 yr.	Normal	0	
CB	F	5 yr.	Normal	0.02	
ML	F	2 m.	Congenital adrenal hyperplasia	0.3-1.9	0.14 (1 week)
LP	F	3 yr.	Congenital adrenal hyperplasia	4.0	0.14 (1 week)
TT	F	4 yr.	Congenital adrenal hyperplasia	4.9	1.57 (1 week)
AA	F	4 yr.	Congenital adrenal hyperplasia	3.8-7.9	0.16 (1 week)
DB	M	8 yr.	Congenital adrenal hyperplasia	2.39	0.58 (10 days)
WD	M	6 yr.	Congenital adrenal hyperplasia	13.9	0.48 (1 month)
SB	M	4 yr.	Congenital adrenal hyperplasia	38.6	6.9 (6 days) 1.6 (10 days)
HB	M	5 yr.	Adrenal tumor	135.7 (no evidence of pregnantriol)	
MD	F	45	Cushing's syndrome	0.06	
LD	F	38	Cushing's syndrome	0.7	

* These results are to be interpreted in the light of the qualifications in the text. The duration of cortisone therapy is indicated for the treatment period.

However by reading over a broad range of the spectrum, it was noted that certain extracts of urine exhibited maximum absorption at 440 mμ instead of 425 mμ, and in general resembled the spectroscopic pattern of pregnantriol. For this reason readings were taken at several wavelengths, particularly at 390, 425, 440 and 460 mμ. Certain of the readings were employed in the formula, as noted, for the calculation of pregnandiol and the optical density at 440 mμ was employed to detect the presence of pregnantriol which shows its greatest absorption at this point instead of 425 mμ. The measure of pregnandiol derived from the above formula and presented in the data must be qualified by recalling the influence of con-

tamination with pregnantriol already described. The correction factor employed would not be expected to eliminate the effects of pregnantriol which may be present in addition to pregnandiol.

Certain of the specimens of urine were extracted with ether and benzene, before and after hydrolysis. The extracts were treated with Girard's reagent and the nonketonic fraction chromatographed on alumina according to Mason (2). Pregnantriol is eluted with 4 per cent ethanol in benzene.

RESULTS

Table I indicates the urinary level of steroids termed "pregnandiol" using the method of Sommerville and Marrian (8). The qualifications for these data have been stated above. Patients with the adrenogenital syndrome due to adrenal hyperplasia uniformly had elevated levels which diminished upon the administration of cortisone. Normal children excreted less than 0.1 mg. per

TABLE II

Effects of ACTH on Pregnandiol Excretion of Two Treated Patients
(Pseudohermaphrodites)

PATIENT	SEX	AGE	PREGNANDIOL (MG. PER 24 HRS. URINE)	
			No. Rx*	with ACTH Rx
		yrs.		
LP	F	3	0.15	1.4 (15 mg. ACTH, IV).
NR	F	6	0.71	27.8 (30 mg. IV)
			0.82	13.9 (3 days later)
				6.35 (4 days later)
				0.40 (4 days after cortisone)

* Both patients had previously been treated with cortisone for approximately two months. Treatment was discontinued three weeks before the above experiments.

day. The single case with a virilizing tumor of the adrenal revealed a very high level.

In previously treated patients from whom cortisone had been withdrawn 2–3 weeks before study, lower levels of "pregnandiol" were found (Table II). The excretion (per 24 hrs.) rose at once after 15 mg. of adrenocorticotropic hormone were given intravenously over a four hour period. In one instance (NR) the level rose to extremely high values.

The observed differences in absorption between pregnandiol and pregnantriol described above were applied to the study of the urine extracts. Specimens from normal adults and children, patients with Cushing's syndrome and a single adrenocortical tumor revealed absorption maxima at 425 mμ with relationships throughout the remainder of the spectrum corresponding to those for pregnandiol. In contrast all extracts from untreated patients with the adrenogenital syndrome showed a peak at 440 mμ which was maximal in

this region and always greater than at 425 mμ (Figure 3). The maximum at 440 mμ (as well as relationships in the lower range) reverted after treatment with cortisone, coincident with the fall in "pregnandiol" and the 17-ketosteroid. The gradual changes in the absorption curves are shown for patient TT in Figure 4. By comparison with Figure 1, it can be seen that the curve changes from one corresponding closely to pregnantriol (especially with reference to 390 and 440 mμ) to one resembling pure pregnandiol. This has been interpreted as indicating the presence of pregnantriol in the extracts before treat-

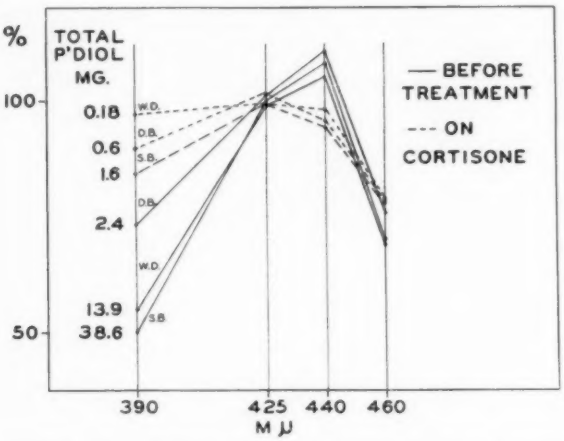

FIG. 3. Absorption curves of urine extracts of three patients with adrenal hyperplasia extracted and purified according to Sommerville and Marrian. Before the administration of cortisone the absorption at 440 mμ is greater than at 425 indicating the presence of pregnantriol. After the administration of cortisone the relative absorption at 440 and 390 compared to the peak at 425 more closely resemble the characteristics of pure pregnandiol. The calculated quantity of "pregnandiol" also diminished after cortisone. The absorption data are charted as per cent compared to the optical density at 425 mμ. All levels at 425 should be 100 per cent but have been spread for clarity.

ment and its disappearance after the administration of cortisone. In patient LP, who had been on treatment (Figure 5, Table II) a single dose of adrenocorticotropic hormone, 15 mg. intravenously over a 4 hour period, raised the excretion of material expressed as "pregnandiol" and restored the peak at 440 mμ which had fallen during therapy. A similar restoration of the peak at 440 mμ was observed after treatment with ACTH in patient NR (Table II). This phenomenon did not occur after the administration of ACTH to two children with normal adrenal function.

Urine specimens from patients WD and LP before treatment were chromatographed in order to isolate pregnantriol. Material eluting as pregnantriol

with 4 per cent ethanol according to the method of Mason (2) was obtained from pools of urine at a time when definite peaks at 440 mμ were present. A 4 day pool of urine of WD yielded 15.5 mg. of material and a 6 day pool from LP, 36.6 mg. The visible spectra of these specimens corresponded to that for pregnanetriol. The identity (L.P. only) was confirmed by infra-red spectroscopy through the kindness of Doctor Harold Mason.

Urine specimens from LP after treatment and from a single normal adult male failed to demonstrate the peak at 440 which is typical of pregnantriol.

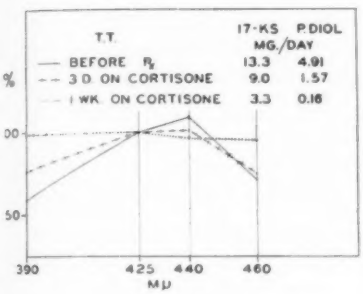

FIG. 4

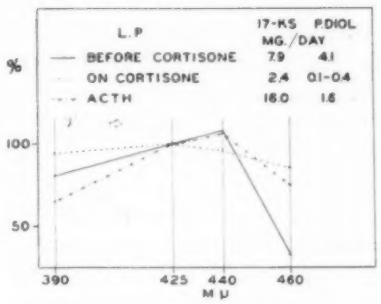

FIG. 5

FIG. 4. Patient TT (pseudohermaphrodite) before, three and eight days after the administration of cortisone. Both the excretion of 17-ketosteroids and calculated pregnandiol diminished with gradual disappearance of the peak at 440 mμ. The tendency of the curve through the regions measured to flatten after one week of cortisone is due to a very low excretion of pregnandiol. The absorption data are charted as per cent as compared to the optical density at 425 mμ.

FIG. 5. Patient LP (pseudohermaphrodite) before the administration of cortisone showing the peak at 440 mμ which exceeds that at 425, which is probably indicative of the presence of pregnantriol. The maximum in this region is altered with the administration of cortisone so that the maximum occurs at 425 mμ. This alteration persisted two weeks after the withdrawal of cortisone but the peak at 440 mμ and relatively lower absorption at 390 mμ was restored after a single dose of adrenocorticotropic hormone.

Chromatography of these same specimens resulted in the isolation of a negligible quantity (less than 0.1 mg.) of amorphous material at the point where pregnantriol is eluted. No specific absorption peaks were obtained with either specimen, nor was any pregnandiol isolated from the urine of LP.

Finally 17-hydroxy-progesterone was administered to normal male adults. In one, after the intravenous administration of 7.0 mg. of 17-hydroxyprogesterone, the urine extracted in the manner for pregnandiol revealed no peak at 440. Chromatography of these specimens did not result in the isolation of material corresponding to pregnantriol. Two normal males were treated with 25 mg. of 17-hydroxyprogesterone injected intramuscularly, and the urines

extracted for pregnandiol. The absorption spectra showed maxima at 425 falling to lower levels at 440 suggesting that little or no pregnantriol was present. Chromatography of the latter specimens did not yield any material corresponding to pregnantriol.

DISCUSSION

The high levels of urinary 17-ketosteroids in the urine of patients with the adrenogenital syndrome and their suppression with the administration of cortisone have been described previously (4). A few earlier studies (5, 6, 7) suggested that the excretion of pregnandiol is also increased in this condition although this was not consistent. The data presented above indicate that such an increase is characteristic of this syndrome, and that pregnantriol is regularly present also. Methods are being devised to better separate these two components since it is possible that the apparent increase in pregnandiol with current techniques, may be in great part due to the presence of pregnantriol. One must conclude that the method of Marrian and Sommerville (8) measures pregnantriol as well as pregnandiol. All of the clinical laboratory techniques available at present for the estimation of urinary pregnandiol are subject to reservations concerning their specificity. This method was chosen, because it requires smaller volumes of valuable specimens of urine, which is a great asset in studying children.

The chromatographic separation of the non-ketonic steroids in four instances with the isolation of pregnantriol when a peak was observed at 440 mμ with the method of Marrian and Sommerville for pregnandiol, justifies the application of this system as a screening technique for the presence of pregnantriol.

It is interesting to speculate on the possible significance of these findings. Hechter (10) has described a schema of biosynthesis for 17-hydroxycorticosterone (compound F) in the adrenal gland. The last step involves the conversion of 17-hydroxyprogesterone to compound F. Jailer (11) has suggested that in the adrenogenital syndrome there is an arrest at this step in the synthesis. The regular presence of pregnantriol (a possible metabolite of 17-hydroxyprogesterone) in the urine of patients with this disease might support this hypothesis. On the other hand on the administration of 7 to 25 mg. of 17-hydroxyprogesterone we did not find any pregnantriol, but this experiment should be repeated with larger doses. Additional doubt is cast by the fact that two patients with the adrenogenital syndrome previously treated with cortisone, showed some increase in the excretion of urinary 17-ketosteroids within 10 days of its withdrawal without a concomitant and comparable rise in the excretion of pregnandiol or pregnantriol. Furthermore in patient NR, when there was a rise in the excretion of pregnandiol and pregnantriol after the administration of adrenocorticotropic hormone there was also a significant increase in the plasma level of compound F (17-hydroxycorticosterone). Further details are forthcoming in a later publication.

CONCLUSIONS

Preliminary studies have established a relatively simple method for the detection of pregnantriol in the urine. It appears that the excretion of pregnandiol is increased and that pregnantriol characteristically appears in the urine of patients with the adrenogenital syndrome due to adrenal hyperplasia. The principle employed in the detection of pregnantriol indicates that the measurement of pregnandiol by the method of Marrian and Sommerville is influenced by the presence of pregnantriol. With the administration of cortisone, the excretion of pregnandiol is suppressed and pregnantriol disappears from the urine. Further studies are contemplated concerning the possible relation of the urinary steroids to the disordered pattern of hormonal synthesis in adrenal hyperplasia.

ACKNOWLEDGEMENTS

The author acknowledges with pleasure the helpful suggestions of Doctors Lawson Wilkins, George Clayton, Jose Cara and Walter Eberlein and the technical assistance of Mrs. Mary Ellen Crafton and Mrs. Betty Lawrence. The figures were kindly prepared by Mrs. Betty Lawrence.

The 17-hydroxyprogesterone and pregnandiol was generously supplied by Doctor H. F. Hailman of The Upjohn Company, Kalamazoo Michigan and Doctor Edward Henderson of the Schering Corporation, Bloomfield, New Jersey.

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BOOK REVIEW

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of the Bulletin)

Ophthalmic Pathology. An Atlas and Textbook. By JONAS S. FRIEDENWALD, HELENOR CAMPBELL WILDER, A. EDWARD MAUMENEE, T. E. SANDERS, JOHN E. L. KEYES, MICHAEL J. HOGAN, W. C. AND ELLA U. OWENS with the editorial assistance of KNIGHT STEWARD. 489 pp., $18.00. W. B. Saunders Co., Philadelphia, Pa.

This volume is the result of the joint efforts of the American Academy of Ophthalmology and Otolaryngology and the Armed Forces Institute of Pathology. It is an outgrowth of an earlier and much smaller volume entitled "An Atlas of Ophthalmic Pathology." It is primarily designed to provide an atlas and text covering the requirements in histopathology of the American Board of Ophthalmology.

This book, however, goes far beyond this primary modest aim. The extensive text, largely the work of Drs. Friedenwald and Maumenee, is primarily a revision and extension of Dr. Friedenwald's older "Pathology of the Eye." In its present form, it is a clear exposition of the currently accepted concepts of ocular pathology. While this book was not designed to be an exhaustive reference work with a complete coverage of the existing literature, nevertheless the ample references to the most important literature bring it close to this rank.

The illustrations, gathered by Mrs. Helenor Wilder from the registry of the Army Medical Museum, are well-chosen, of remarkable excellence and almost completely cover the entire field of ophthalmic pathology. The half-tone reproductions are all that could be hoped for, the format of the volume is pleasing and the print excellent. This volume has been long awaited, and now that it is finally available, it fulfills all expectations. The authors, the contributors and the sponsors are to be congratulated on a task well done.

A. C. W.

Surgery of the Eye, 3rd edition. By MEYER WIENER and HAROLD SCHEIE. 449 pp., $15.00. Grune and Stratton, Inc., New York City.

The third edition of this standard text-book on ocular surgery follows closely the plan of previous editions and maintains the same clarity of description. The first three chapters deal with the general subjects of basic techniques in ocular surgery, anesthesia, preoperative and postoperative care. A description of cataract operations follows. Most of the

discussion in this chapter describes the authors' technique using a sutured scleral flap supplemented by scleroconjunctival sutures. However, other widely used techniques are mentioned. The next chapter covers the surgery of glaucoma. The classification of the operations for glaucoma and the discussions of the surgical treatment of glaucoma are especially good. The authors' technique for retinal detachment operations follows. There are chapters on the surgical methods for removing intraocular foreign bodies, operations on the cornea, operations for the removal of the eye, and operations on the conjunctiva. The sections dealing with the plastic procedures used on the lids and sockets are excellent. The book ends with descriptions of the methods used for operating on the extraocular muscles and for operations on the tear apparatus.

The fine drawings are one of the features of this edition, as of the previous editions of this book. They present in clear and graphic fashion, the necessary steps in each of the operations described. No attempt has been made by the authors to discuss comparatively the many operative techniques that have been described for the treatment of various ocular conditions. They have selected the methods which they judge to be the best for a given condition.

This new third edition will be found as useful and valuable to the practitioner or student of ophthalmology, as have the previous editions.

WILLIAM C. OWENS

Rheumatic Diseases. Diagnosis and Treatment. By EUGENE F. TRAUT. 942 pp., $20.00. The C. V. Mosby Co., St. Louis, Missouri.

This book presents an extensive discussion of the rheumatic diseases. Its best sections are those devoted entirely to diagnosis and treatment. In these the author has had the benefit of his own clinical experience. Other portions, particularly those on etiology, include considerable data which are presented in such an uncritical fashion as to make the information only confusing. The book lacks the critical approach of a text-book and the comprehensiveness of a review.

F. F. SCHWENTKER

Physiologie Normale et Pathologique du Metabolisme de L'eau. By JEAN HAMBURGER and GEORGES MATHÉ. 502 pp., 2.850 francs. Editions Medicales Flammarion, Paris, France.

In the past decade new techniques and new concepts have resulted in a great expansion of interest in the problems of the water and electrolyte economy of the body. The considerable number of monographs and reviews dealing with this branch of clinical physiology which have appeared within the past few years was therefore to be expected. This volume by Drs. Hamburger and Mathé is ambitious in scope, and attempts a comprehensive historical review of the literature with a critical summary of the current ideas concerning the physiology of the body water and an analysis of the disturbances resulting from disease.

The book is divided into three main parts: The first section deals with the physiological mechanisms which regulate the water balance of the body and the exchange of fluids within the body. The authors include detailed summaries of their own studies in this field particularly those relating to the movement of water between cells and extracellular fluid resulting from variations in the osmotic pressure of extracellular fluid. Some of these studies were made in human subjects using the interesting technique of intestinal perfusion to modify the composition of extracellular fluid.

Part 2 consists of a summary of the technical procedures available for measuring the water content of the body and the various compartments of body water. The methods are given in considerable detail but this section can not be used by the uninitiated without considerable supplemental information. Some of the techniques have only historical interest and in

general one may not get a true idea of the theoretical and practical difficulties involved in measuring the so-called extracellular fluid space of the body.

Part 3 is devoted to the disturbances of water balance and distribution resulting from disease. The most interesting section deals with what the authors term "dyshydration" i.e. disturbances of osmotic pressure relationships between cells and extracellular fluid, which are more thoroughly discussed than in most reviews of this subject. The sections on treatment outline principles of management in rather general terms. The authors use intestinal perfusion as a method of therapy to an extent which is not common in this country.

To the clinician and physiologist interested in problems of the physiology of the body water the review of the literature and the extensive bibliography of over 2,500 references should be valuable particularly since the European literature is cited to a greater extent than in most American treatments of this subject.

HAROLD E. HARRISON

Diseases of Metabolism, 3rd edition. Edited by GARFIELD G. DUNCAN. 1179 pp., $15.00. W. B. Saunders Co., Philadelphia, Pa.

The first edition of this book appeared in 1942, the second in 1947, and the third in 1952. Dr. Duncan and his coauthors are to be commended for undertaking the laborious task of bringing *Diseases of Metabolism* up to date every five years. Their efforts have been made more difficult because the field of metabolism has been developing so rapidly in recent years. Since the subject matter treated in each chapter has such a broad scope, it has been necessary to present the material in a condensed but lucid fashion. This the various contributors, who for the most part are established authorities in their respective fields, have accomplished admirably.

Compared to the previous edition, the names of several new authors appear, and the chapters dealing with Carbohydrate Metabolism, Water Balance in Health and Disease, Undernutrition, Diseases of the Thyroid, and Diabetes in Children have been completely rewritten. Drs. Soskin and Levine have expanded the section on the intermediary metabolism of carbohydrate to include most of what is known of the biochemical reactions concerning the oxidation of glucose by tissues. The role of endocrine factors in the regulation of carbohydrate metabolism is expounded in greater detail than in the previous edition, and the authors have emphasized the functions of the liver in the control of the level of the blood sugar. Dr. Peters has enlarged the chapter on Water Balance in Health and Disease to broaden our understanding of the basic physiological alterations in clinical states in which balances of water and electrolytes are disturbed. In the chapter on Diseases of the Thyroid, Drs. Rawson and Rall have presented the newer concepts in the physiology of the thyroid gland, and have considered the uses of radioiodine in diagnosis and therapy. In this edition several other new topics are discussed such as NPH insulin, vitamin B_{12} and folic acid, and ACTH and cortisone. The chapter in the previous edition on hematologic disorders has been omitted, but a new one on porphyrin metabolism has been inserted.

In most instances the information presented is well documented so that the reader can refer to original papers on controversial subjects. In some cases there is repetition of material but this is practically unavoidable since the different authors attempt to deal with all aspects of their topics. With a few exceptions, the latest information on both the physiological and clinical views of the subjects discussed is included. A single volume dealing with all phases of metabolism could not possibly be complete in all details. Nevertheless *Diseases of Metabolism* should be invaluable to the student, the research investigator, and the practicing physician, and it remains one of the best text-books in the field of medicine today.

JOHN G. WISWELL

BOOKS RECEIVED FOR REVIEW

Anzeige und Ausführung der Eingriffe an Ohr, Nase und Hals. By WALTHER UFFENORDE, HELLMUT UFFENORDE AND HORST WULLSTEIN. 540 pp., Ganzl. DM 79.80. Georg Thieme Verlag, Stuttgart, Germany.

Blood Clotting and Allied Problems. Transactions of the Fifth Conference, January 21 and 22, 1952. Edited by JOSEPH E. FLYNN. 368 pp., $4.95. Josiah Macy, Jr. Foundation, New York.

Das Inselsystem des Pankreas. Entwicklung, Histobiologie und Pathophysiologie. By HELMUT FERNER. 186 pp., Ganzl. DM 29.70. Georg Thieme Verlag, Stuttgart, Germany.

Das Phasenkontrastverfahren in der Medizin. By HANS-GEORG HANSEN, ALEXANDER ROMINGER AND KURT MICHEL. 92 pp., Vandenhoeck & Rupreche. Göttingen, Germany.

Die Alkoholhalluzinosen. By G. BENEDETTI. 58 pp., Kart. DM 7.50. Georg Thieme Verlag, Stuttgart, Germany.

Die Angiographie zur Erkennung, Behandlung und Begutachtung peripherer Durchblulungsstöneugen. By H. H. PÄSSLER. 115 pp., Kart. DM 29.70. Georg Thieme Verlag, Stuttgart, Germany.

Die Neurose als Reifungsproblem. By WOLFGANG KRETSCHMER. 92 pp., Kart. DM 10.80. Georg Thieme Verlag, Stuttgart, Germany.

Don't Be Afraid of Your Child. By HILDE BRUCH. 297 pp., $3.75. Farrar, Straus and Young, Inc., New York City.

Grundriss der Elektrokymographie. Phasenanalyse und Raumkymographie des Herzens. By K. HECKMANN. 36 pp., Kart. DM 6.60. Georg Theime Verlag, Stuttgart, Germany.

The Literature on Streptomycin 1944–1952. By SELMAN A. WAKSMAN. 553 pp , $5.00. Rutgers University Press, New Brunswick, New Jersey.

Klinische und sprachwissenschaftliche Untersuchungen zum Agrammatismus. By G. KANDLER AND A. LEISCHNER. 72 pp., Kart. DM 6. Georg Thieme Verlag, Stuttgart, Germany.

Metabolic Interrelations With Special Reference to Calcium. Transactions of the Fourth Conference, January 7 and 8, 1952. Edited by EDWARD C. REIFENSTEIN, JR. 262 pp., $4.50. Josiah Macy, Jr. Foundation, New York.

Operating Room Technic. St. Mary's Hospital, Rochester, Minnesota, 4th edition. By Sisters of St. Francis of St. Mary's Hospital. 345 pp. W. B. Saunders Co., Philadelphia. Pa.

Problems of Consciousness. Transactions of the Third Conference, March 10 and 11. 1952. Edited by HAROLD A. ABRAMSON. 156 pp., $3.25. The Josiah Macy, Jr. Foundation. New York.

Protein Metabolism, Hormones and Growth. A Symposium. Edited by WILLIAM H. COLE. 80 pp., $1.50. Rutgers University Press, New Brunswick, N. J.

Psychische Komponenten der Sinnesorgans. Eine Psychophysische Hypothese. By BERNHARD RENSCH. 200 pp., Kart. DM 22.50. Georg Thieme Verlag, Stuttgart, Germany.

Textbook of Surgery. Edited by H. F. MOSELEY. With a Foreword by G. GAVIN MILLER. 896 pp., $15.00. The C. V. Mosby Co., St. Louis, Missouri.